

Time for a Cod Peace

THE cod war took a turn for the worse last week with a British trawler being holed by fire from an Icelandic gun boat. Relations between Britain and Iceland are now strained to their utmost and it is clear that the sooner negotiations are resumed between the two countries the better it will be for both governments and fishermen. In the circumstances it is unfair for Iceland to insist that the British navy departs before talks restart.

The issues are clear enough at this stage and Iceland's demand to control the fishing within fifty miles of its own coast is mostly a means to ensure, as best it can, the future economic growth of the country. It is agreed that the cod in Icelandic waters is fully exploited and for Iceland itself to obtain greater benefit from the seas nearest to its shores, other countries must restrict their catches in these waters.

The British fishermen see this as yet another attempt to deprive them of good fishing grounds. About a third of all the cod brought into British ports in 1971 was obtained from the waters around Iceland and in contrast to other areas in the North Atlantic where cod is traditionally found—such as the North East Arctic region—the waters are not at present over fished. There is little hope of obtaining cod further than fifty miles from Iceland for most of the cod is found on the hundred fathom deep continental shelf within the fifty mile limit.

Cod fishing in the north Atlantic is controlled by two commissions, the International Commission for North West Atlantic Fisheries and the North East Atlantic Fisheries Commission. Britain and Iceland are members of both bodies. Part of the present troubles stems from the decision of the North West Commission to limit the catch in the waters under its control, not by the traditional method of altering the size of the fishing net mesh but by imposing a limit on the catch. These controls only affect the fishing off the west coast of North America at present. These controls were introduced at the beginning of 1973 but the efforts of the North East Commission to introduce similar restrictions have been thwarted by no other country than Iceland which only two weeks ago refused to accept a similar agreement drawn up by the North East Atlantic Fisheries Commission.

These developments, coupled with the introduction in recent years of factory ships—that is trawlers with refrigerated storage—have convinced the Icelandic Government that the waters near its own shores would be the first to be put under pressure if tight limits were imposed in the North East Atlantic, in particular the seas to the north of Norway and to the south of Spitsbergen and the Barents Sea, which have been over fished.

But is the cod in danger of being fished to extinction in the North Atlantic? Clearly the cod is not threatened to the same extent as the whale for example. Whales reproduce once or twice every one or two years but a large cod could lay several million eggs during its lifetime. There is a need for only two of these eggs to reach maturity to ensure that the stock is replaced. The size of the mesh now used by cod fishermen—130 millimetres—ensures that no cod is caught until it is at least two years old and the rate

of fishing is such that it is estimated that about fifteen per cent of two year old cod will survive for seven years, the age of full maturity. But, it must be said, there is a fine line between fishing enough to ensure that the stock is replaced and fishing to ensure that the stock will not become depleted. At present the North West Commission is advised by scientists as to the amount of fishing that can be done and the commission then divides this up among the representative countries according to the amounts of cod which they landed during previous years. What is at once clear is that the anomalous situation with only one of the fishing commissions setting limits on catches will impose a great deal of pressure on the fishing grounds under the aegis of the other commission and, whatever happens about Iceland's claims, the two commissions should, at least, present a common policy on catches. In particular setting a limit on the cod that can be caught in the Arctic regions north of Norway will ensure that these grounds will recover properly and will not be over fished again during the recovery period.

But how will this help settle the dispute over fishing within fifty miles of Iceland's shores? The Icelandic waters are, by mutual agreement, fished as much as is wise already. Setting quotas on the fish taken from these waters will not necessarily alter the total catch. More evidence is needed soon to decide whether Icelandic waters are being overfished or not.

It must be faced that Iceland badly wants to improve its economy. Fishing is the country's chief industry and an improvement in this sector can only be obtained by an increased catch which, without seriously damaging the stock, can only be achieved at the expense of another country's catch. Can a way now be found over the negotiating table to aid Iceland without seriously damaging the livelihood of the British trawlermen?

100 Years Ago



PROFESSOR WYMAN has concluded, as the result of explorations among the shell mounds of Florida, U.S., during the past winter, that the aborigines by whom they were constructed must have been decided cannibals, as in eight different instances he has found considerable quantities of human bones in the shell heaps, the bones themselves being broken up and split, just as in the case of the bones of other animals. This, he is satisfied, was not the result of burial, but was done for the purpose of obtaining the marrow, probably after the flesh had been devoured.

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OLD WORLD

"European Science Foundation Inevitable" Sir Brian says

A DEAL of European banner waving was apparent in Berlin last week when Sir Brian Flowers, Chairman of the Science Research Council, delivered the Queen's lecture and announced that "I now believe that the creation of a European Science Foundation is not only desirable but inevitable and that it will happen quite soon".

Sir Brian, who declared that he was out to be "frankly political" although "only in the context of European Science", outlined how he thought the new foundation should work and what it should do.

The foundation was originally proposed in its present form by Commissioner Spinelli of the European Commission in June last year as a body "that would not be designed as a substitute for existing centres and associations, but to support their actions and contribute to their effectiveness by way of coordination". Its particular tasks would be to ensure the mobility of research workers, to organize international meetings, support European activities and projects, finance laboratories that have the potential of being European centres of excellence and examine national plans for large research facilities with a view to their being funded on a cooperative basis in the future.

Sir Brian clearly approved of these aims, but he went on to underline certain points he feels to be important. Membership of the foundation must not be limited to members of the community, he said. "Scientific cooperation cannot be limited by national borders" and limiting cooperation could submit fundamental science to political and economic goals which are often too immediate to be relevant to scientific and cultural pursuits.

The foundation must also be autonomous. Finance must come both from the community and from the national organizations that adhere to it, and its scientific judgements and policies must remain in the hands of the scientists themselves. Equally, Sir Brian said, the initiatives must come from outside the foundation rather than from its bureaucracy. As the Max Planck Society has put it, fundamental research "should rather be supported than organized, and rather promoted than programmed".

To help ensure this the commission's share in the foundation, important though it is, should not be too great. More than half the finance should come

from national organizations such as the research councils.

The foundation's budget should therefore be—at least at the beginning—a small one. "Major expenditures" Sir Brian said, "should come, as they do now, from coordinated national funding". This will avoid the twin dangers of financing projects purely for political reasons "merely in order to appear European", and of supporting big science (space research, astronomy and particle physics) which can only be funded internationally, to the detriment of little science such as botany, which can be funded locally.

BACTERIOLOGY

What's in a Name?

THE nomenclature of bacteria is likely soon to be revised. After five years work a committee of bacteriologists has produced a series of proposed revisions to the International Code of Nomenclature of Bacteria¹ that could result in ninety per cent of the published names for bacterial taxa disappearing by 1980 as a new list is drawn up.

This somewhat extreme solution, however, is really only a measure of the problems that face bacteriologists. The International Code of Nomenclature of Bacteria lays down that taxa must always be known by their earliest name, with 1753 being taken as the earliest acceptable date. This has led to lengthy searches in dusty literature by systematists who have to check that they have found the earliest name for a group of organisms. But, not surprisingly, the first descriptions of micro-organisms are often so poor by modern standards that their identity now is a matter of opinion rather than of fact, and a number of names are being conserved by international agreement when all they are doing is creating work for the people the code is meant to help.

The solution that has been proposed by a five man committee headed by Dr S. P. Lapage of the National Collection of Typecultures is that between now and 1980 a list should be prepared of all names for bacterial taxa that have any claim to be worth keeping. The remainder—perhaps ninety per cent of the old names—will be forgotten. Thus if no one cares enough about an old name for a species that in fact cannot now be clearly recognized, it is better to forget it and allow it to be redis-

¹*International Journal of Systematic Bacteriology*, **23**, 83–108.

Sir Brian was equally adamant that cooperation should take place on the small as well as the grand scale. University departments across Europe should cooperate with each other, and indeed "the research councils are already trying to encourage such developments".

The other key role that Sir Brian envisages for the foundation is that of supporting European learned and professional societies. Citing the example of the European Physical Society, which is currently short of cash, Sir Brian said that the relatively trivial sums required to finance such bodies are proving hard

covered and renamed if need be. By 1980 only the names on the new list will be used.

In an attempt to prevent a similarly chaotic situation building up in the future, Dr Lapage and his committee have also recommended that any new names must be published in the *International Journal of Systematic Bacteriology* if they are to be valid.

Since, from 1980, the new official list will be published in that journal, this means that all these and all future names will be readily available in one publication. Clearly the committee cannot legislate that all descriptions of new taxa must appear in one journal, so a distinction that already exists in the code between "effective publication" and "valid publication" has been exploited. "Effective publication" is publication of a description anywhere in the scientific literature, but for it to be "valid" the name must also be published in the *International Journal of Systematic Bacteriology* with a reference to the description if that has been published elsewhere.

At first sight the solution seems neat and elegant. In spite of the size of the problem, bacteriologists are fortunate in having a relatively small number of species to manage. Botanists and zoologists have similar problems, but the range of species involved may make a similar solution impracticable.

Comments on the new proposals will be considered during the summer and the revised code will go before the International Congress of Bacteriology for approval this autumn. Assuming the congress approves, bacteriologists may, by 1980, be able to spend less time combing dusty shelves.

to find from national sources and should be made available through the foundations.

The foundation should also be given the task of fostering European professional institutions as well as learned societies. Such bodies should help establish confidence in the professions, and would, Sir Brian implied, go a long way to solving the problem of what qualifications can be recognized throughout Europe—a problem with which the Commission has struggled long, hard and fruitlessly.

But before outlining his ideas on how a European Science Foundation should operate, Sir Brian pointed the lessons that can be learnt from Europe's attempts at scientific collaboration over the past twenty years.

To start with, Sir Brian said, the science to be undertaken must be good science. The initiative for the new organization must come from scientists, not politicians, and the objectives must be clearly defined. Particularly in technological fields the overall policies within which the aims of the organization are set must be clearly understood—"there should be no projects without policy as was the case when Euratom was created".

Further, the cost or content of programmes must justify the cooperation. "It is not enough that we have problems in common; we have to show that we are better able to solve these problems jointly rather than separately".

Once a European organization is set up, however, each country "should give its support of that organization priority over any residual national effort in this field . . . national programmes should wherever possible exist, but they should be supportive or complementary, and clearly seen to be so".

Sir Brian also underlined the all too familiar dangers of a rigid policy of "juste retour", whereby each country expects to recover, through contracts placed within its national industries, the money it has put into cooperative organizations. The policy is "nothing but a wasteful irritation", Sir Brian said. "With the growth of European companies it is in any case likely to become an irrelevance of no economic significance and of dubious political value".

TELEVISION

Angels Not Apes

DR JACOB BRONOWSKI'S television programmes on the BBC are certain to be a sensation. The title, *The Ascent of Man*, shows where he stands. Not for Bronowski a downbeat Darwinian word like descent. Instead, he is ebulliently, unfashionably but not outrageously anthropocentric. The first of the pro-

grammes, three weeks ago, put the point directly. *Australopithecus* was above all intelligent, and intelligence made it possible for even australopithecines to invent cultural adaptations to different kinds of habitat. The result is that their offspring genera have inhabited the world and that the Laplanders whom the BBC has taken the trouble to film exquisitely are intrinsically smarter than the reindeer on which they live, partly as nomads and partly as neolithic agriculturalists. Dr Bronowski's illustrated hymn to the cleverness of people is in part at least intended as challenge to the contemporary mood of those who hold that all species are intrinsically equal, like numbers in a Bingo game. It is an eloquent challenge. Altogether there will be thirteen programmes. The film is everywhere superb. So, in his idiosyncratic way, is Bruno, as they call him. Alone among television performers, he has made the pause for words a source of tension and a vehicle for excitement. And, as always, he takes the grand view. The BBC has labelled this series of films *A Personal View* and that is understandable, not necessarily a piece of cowardice. For many of Bronowski's messages are open to argument. What matters is the grand conception and there is no doubt that the BBC now thinks, with some reason, that it has on its hands a fitting counterpart to the series of films by the then Sir Kenneth Clark labelled *Civilization* which omitted to mention technology other than that invented by Leonardo da Vinci.

In principle, the BBC is right and the new series is a triumph for it as well as for Bronowski. Will the quibbles that can be raised become the scenario for another series? Precisely how did the *Australopithecines* escape from East Africa (a programme of research, not a question for the BBC)? Might it not have been sharper, in the third programme, concerned to show how building has been a constant outlet for inventiveness, to have played church music for the Watts towers and George Gershwin for cathedrals (a question for the BBC, not for Bronowski)? But even if it is right, as the eleventh programme will in due course argue, to infer from the uncertainty principle and the doctrine that nothing is absolutely knowable that people should be tolerant of other people's ideas and even other people, is there not some logical gap to be bridged?

Where does Bronowski stand on Jensen-Eysenck? And may there not be more to be said about tolerance (and the need of it) than can be done knee-deep in a mud of funerary ashes in an Auschwitz pond (which is a question for Bronowski)? That said, the programmes are enough of a sensation for *Private Eye* already to have begun to mock them and they will make a mark.

DRUGS

Safety Suggestions

RECOMMENDATIONS for international drug safety legislation have been formulated as a result of a conference held in Italy recently. The special commission on internal pollution, a collection of scientists representing most of the countries in the European Community, was organized by Sir Rudolph Peters, University of Cambridge, who took the chair. Mr Jean Rey, the Belgian Minister of State and recently president of the EEC, proposed that Brussels should be approached unofficially to consider establishing a voluntary body to harmonize drug safety evaluation within the community.

The recommendations made include the development of clinical pharmacology, which in many EEC countries is not regarded as a speciality, and a greater degree of integration between animal and human studies. Compounds should be considered for study in people as soon as experimental work indicates potential therapeutic value and a lack of significant side effects. Human studies should then dictate subsequent animal experimentation.

Value Added Science

FINANCIAL musical chairs between the Treasury, the research councils and their customer departments have marked the introduction of the financial changes promised in last July's White Paper on government research and development which came into effect on April 1—the same day as Mr Anthony Barber's Value Added Tax.

The Department of Education and Science's budget was reduced by the Treasury by the amounts that the three research councils involved in the Rothschild cuts were due to lose to their customer departments. In the case of the Agricultural Research Council this amounted to £6.5 million which the Treasury gave instead to the Ministry of Agriculture, Fisheries and Foods. However, as the ministry and the ARC have not yet agreed which projects the ministry is to count as part of its contract research with the ARC, MAFF simply transferred the £6.5 million straight across to the council. Only by this time the £6.5 million had become £7.2 million as the council has to charge the ministry VAT as contract research is a service. The extra £0.65 million will shortly be transferred back to the Treasury which provided the money to MAFF in the first place. It keeps the civil servants busy.

NEW WORLD

Cries of Distress from the Medical Schools

by our Washington Correspondent

For the past few years medical schools in the United States have been caught in a financial squeeze and there has been persistent talk of budgetary crises. But the talk turned to cries of anguish in January this year when President Nixon's budget for 1974 was published—for the first time, the schools are faced with an absolute cut in federal funds, and with the abolition of some cherished programmes. Exactly what effect the proposed cuts will have on the medical schools has, however, been a matter of some debate, and so the Association of American Medical Colleges (AAMC) has conducted a survey of its members to find out. The results, published last week, confirm the worst.

According to replies from 78 of the 113 medical schools in the United States, the proposed funding cuts will result in a reduction in staff of at least 1,400—one out of twelve faculty members—possible reduction in enrolments, curtailment of some programmes designed to assist minority and disadvantaged students and a variety of other cost-cutting measures. Small wonder that Dr John A. D. Cooper, president of the AAMC, last week called the proposed 1974 budget "a serious blow to medical education, biomedical research and health care in this country".

Although the medical schools receive funds from a variety of sources, they have become increasingly dependent on the federal government for their money: in 1972, for example, the government provided 44 per cent of the total income of the medical schools. But dependence on federal financing has made the schools particularly susceptible to vagaries in the budget, clashes between Congress and the White House and the whims of officials in the Office of Management and Budget.

In the early 1960s, their finances were relatively healthy, largely because of the rapid increase in the budget of the National Institutes of Health and the consequent flow of research money into the universities and their medical facilities. But the schools' troubles began with the levelling off in research funds of the late 1960s and early 1970s, went from bad to worse when Congress and the White House failed to reach agreement on the appropriations bill for the Department of Health, Education and Welfare last year (see *Nature*, **242**, 81, 1973), and will deteriorate even further if the Administration's plans for next

year are carried out. The schools' financial ills are also exacerbated by the fact that they have nearly all been increasing their intake in response to present and projected shortages of doctors.

President Nixon's budget for 1974 provided a double blow to the medical schools, for not only did it propose to cut back on spending in 1974, but it also outlined cuts that the Administration intended to make in its own estimates for 1973. The schools are therefore faced with an immediate cut that they had not anticipated, and the prospect of much less money in 1974 than they had bargained for. According to the AAMC survey, the 1974 funds proposed in the Administration's budget are at least 25 per cent less than the schools planned for.

Among the programmes which the Administration plans to cut or abolish, training grants and fellowships, and research grants and contracts from the National Institutes of Health (NIH) constitute the biggest loss to the medical schools. According to the AAMC survey, the 78 medical schools which took part estimated before the Administration's budget was published, that they would receive \$599 million from these programmes in 1974, but the budget only contains \$465.3 million—23 per cent less than they had planned for. The schools are also suffering from abolition of regional medical programmes and savage cuts in special project grants and general research support grants. One effect of the cutbacks at NIH is that the support of new applications—as opposed to continuing the funding for present projects—will decline by 40 per cent.

According to the AAMC survey, these will be the chief consequences of the cutbacks:

- About 8 per cent of the faculty members and 15 per cent of supporting staff of US medical schools will have to be discharged unless alternative sources of funding can be found
- Curriculum innovations, such as early clinical experience and shortened courses, will suffer
- The abolition of Regional Medical Programs will, according to Dr Cooper, "seriously curtail the ability of the medical schools to become involved in community health programmes"
- One-third of the schools responding to the survey indicated that they may have to reduce the size of the enrolments, and some have already decided to do so
- Some entire research programmes will be stopped.

What are the chances that at least some of the money will be restored to the health budget? There is little doubt that Congress will try to keep the abolished programmes alive, and that it will substantially increase the President's budget request. But the problem is that the White House is still holding the whip hand in budgetary matters. In the present fiscal year, the Office of Management and Budget simply refused to spend several hundred million dollars which Congress had appropriated for various programmes because the Administration wanted to cut down on federal expenditure. Although that precipitated a bitter power struggle between Congress and the White House over which branch of government con-

Shuttle Wins, Science Loses

THE space shuttle easily survived its first Congressional hurdle this year when the House of Representatives last week passed the NASA authorizations bill. An amendment to the bill, seeking to delete the \$500 million dollars earmarked for the shuttle, was easily defeated by 95 votes to 20. Congressional critics of the project have three more chances to get rid of the shuttle during the next few months, when the authorizations bill comes before the Senate and when the appropriations bills are considered by each house.

As far as the rest of NASA's budget is concerned, the Committee on Science

and Astronautics, which drew up the authorizations bill, reduced the budget request for physics and astronomy by \$5 million, and the request for lunar and planetary sciences by \$2 million. The money saved from these programmes should be added to the Earth Resources Technology Satellite programme, the committee suggested. The effects of the cuts on space science—if they are agreed to by the Senate—are not yet known, but it is possible that the Atmospheric Explorer satellite may be delayed by about 6 months. But, at least at present, no senior challenge seems to be facing the shuttle.

trols the pursestrings (see *Nature*, **241**, 81; 1973), the issue has not yet been resolved. It can thus safely be assumed that President Nixon will veto the HEW appropriations bill again this year if he considers it inflationary or, failing that, the OMB will simply not spend any extra money that Congress may add to the Administration's budget request.

Senator Edward Kennedy, the chairman of the Senate Health Committee, said in a speech on the NIH campus last week that "I will do everything in my power to reverse these short sighted health policies". In particular, he announced that he will soon introduce legislation which will change the statutory basis of the training grant programme, in an effort to force the Administration to keep the programme alive. Bills extending the programmes which the Administration wants to abolish are also in various stages of passage through the Congressional mill.

The Administration's response to the cries of anguish from the medical schools is to stand firm. The general principle that the Department of HEW has been propounding is that the federal government should not be in the business of directly supporting young scientists through training grants, but that the future supply of medical scientists should be left to market forces to determine. At present, the Administration argues that the US is overproducing medical scientists and in testimony before Senator Kennedy's subcommittee, for example, Caspar Weinberger, Secretary of HEW, has called the protestations of the medical schools and scientists "predictable".

PEER REVIEW

Open Attack

by our Washington Correspondent

FEW things affect the biomedical community more directly than the way in which the National Institutes of Health disburses grants and contracts to medical researchers. It is thus not surprising that recent rumours and press reports indicating that the Administration is launching an attack on the very basis of the NIH granting system have been greeted with misgivings and alarm. The subject of the rumours and reports is a memorandum which has been circulating among top NIH officials for the past few weeks. Prepared by the Office of Management and Budget (OMB), the contents of the memorandum have been kept tightly guarded, but last week a copy found its way into the hands of Senator Edward Kennedy and he duly made it public.

It seems clear from the document that the biomedical community has every reason to be alarmed. The focus for the Administration's attack is the

peer review system by which grant applications are evaluated, and the memorandum suggests eight ways of "improving" it. They range from total abolition to relatively minor tinkering, but the thrust of the document is towards more targeted research and less of what the Administration has taken to calling "investigator-initiated" research.

The peer review system lies at the heart of the NIH granting process, and although nobody has suggested that it is entirely flawless, it is vigorously defended within NIH and in the rest of the biomedical community. It works like this. A grant application is initially examined by a study group of between 12 and 15 practising scientists working in the same field as the proposal. The study group approves or disapproves the proposal, assigning it a priority rating based on its scientific merit. The next stage is review by a national advisory council, which screens the proposal for policy and programme focus. Each institute of NIH has such an advisory council, consisting of scientific and lay members, and these councils pass recommendations on to NIH staff who in theory retain the final decision on which projects should be funded. In the past fiscal year, some \$1,481 million worth of grants and contracts came under the scrutiny of peer review groups in NIH and in the National Institute of Mental Health.

What disturbs the management types in the Office of Management and Budget is that the process seems to give little administrative control to officials of NIH or the Department of Health, Education and Welfare and that makes it difficult to target or direct research. To back up its case against the system, the OMB cites a number of unidentified studies made by the Executive Branch and by Congressional committees. The criticisms which the OMB says have been made of peer review fall generally into three categories. The first is the difficulty in gaining administrative control over the allocation grants, the second is the conflicts of interest which may arise when scientists who are members of study groups "determine the allocation of research funds that they also receive", and the third is that the system defines scientific merit or quality for itself, and is thus "not subject to objective measures of assessment".

Among the eight possibilities for changing the peer review system set out in the OMB memorandum are the following. The most drastic is total abolition of the system and complete reliance on administrative review—the memorandum describes such a step as "the ultimate in return to administrative control". But that would require legislation to remove statutory requirements for national advisory committees to approve grants and contracts. Less

drastic are suggestions which would involve retaining the advisory councils, but which would abolish the study groups. In their place, the memorandum suggests that NIH staff could be used for initial review of grant proposals, that consultants could be called in on an *ad-hoc* basis, or that reviews could be conducted by mail by randomly selected anonymous reviewers. To circumvent charges of conflict of interest, the OMB suggests that members of study groups should be changed every two or four sessions, and it also floats the idea of prohibiting members of advisory committees from receiving NIH research funds while serving on the committees or for a time afterwards.

The memorandum is now doing the rounds of NIH officials who will submit their comments to Dr Charles C. Edwards, the Assistant Secretary of HEW for Health. There is little doubt that the institutes will resist fiercely any attempt to scrap peer review entirely, and it seems that in any case there is little chance of that happening. If such a drastic step were taken, it would create much more of an uproar in the biomedical community than even the outcry that has greeted the recent cut-backs in funding and the abolition of training grants.

But, in the context of recent statements by Caspar Weinberger, Secretary of HEW, the trend towards more contract research in NIH, and the general policy of the Administration to concentrate on scientific research likely to have short term payoff rather than on more basic research, some alteration of the peer review system and tighter management control of biomedical research seems clearly on the cards. Weinberger said recently that he wants to "redress the balance" in research funding—"there has been too much emphasis on investigator-initiated research and not enough on directed research".

Members of the biomedical community are concerned, however, that the trend towards targeted research—as exemplified by the cancer programme—is going ahead at the expense of more basic research which could well produce key leads in the drive to understand the mechanism of diseases. The threat to peer review is one aspect of such a trend.

At that meeting, the Council of the Institute of Medicine instructed its President, Dr John R. Hogness, and the President of the Academy itself, Dr Philip Handler, to urge the Administration not to tamper with the peer review system without a thorough study. Congressional backing is also in sight for peer review, for Senator Edward M. Kennedy said last week in a speech on the NIH campus that he will soon introduce a bill to strengthen the system.

NEWS AND VIEWS

Muscular Dystrophy and the Neurogenic Hypothesis

MUSCULAR dystrophy in man has long been regarded as a primary degenerative myopathic process. Histological study of the tissues in such cases coming to autopsy has generally shown that the central nervous system is intact and that the abnormal pathological changes are confined to the voluntary muscles. Recently, however, it has become clear that many patients diagnosed clinically in the past as suffering from muscular dystrophy, particularly of the limb girdle and facioscapulohumeral varieties, may be suffering from a spinal muscular atrophy (the so-called Kugelberg-Welander syndrome). In such cases it is now well recognized that the examination of skeletal muscle specimens obtained by biopsy may reveal so-called secondary myopathic change (Drachman, Murphy, Nigam and Hills, *Arch. Neurol. (Chicago)*, **16**, 14; 1967; and Mastaglia and Walton, *J. Neurol. Sci.*, **12**, 15; 1971). It has, however, been generally agreed until recently that the most severe form of muscular dystrophy, namely, the X-linked Duchenne variety, which usually produces clinically evident difficulty in walking by the age of 3 years, inability to walk by the age of 10 and death by the age of 20, is still a primary myopathic process. Nevertheless, it is well recognized that the average intelligence quotient in such patients is lower than in a control group of male children of comparable age, and Rosman and Kakulas (*Brain*, **89**, 869; 1966) demonstrated neuronal heterotopias in the brains of some such patients at autopsy.

Within the past few years much fundamental work in relation to the pathogenesis of muscular dystrophy has been carried out in animal models, especially mice of the Bar Harbor (129/REJ/dy) strain and in homozygous dystrophic New Hampshire chickens (line 304) (Julian and Asmundson, *Muscular Dystrophy in Man and Animals*, pp. 458-498, Karger, New York, 1963). In 1967, McComas and Mrozek (*J. Neurol. Neurosurg. Psychiat.*, **30**, 526) carried out microelectrode studies from single muscle fibres in dystrophic mice of the Bar Harbor strain and found that approximately one-third of these were functionally denervated. In such mice, electrophysiological studies carried out subsequently by Harris and Wilson (*Nature*, **229**, 61; 1971) showed that the number of motor units was reduced, but the surviving units were still of normal size. Furthermore, Jedrzejczyk, Wieckowski, Rymaszewska and Barnard (*Science*, **180**, 406; 1973) found in dystrophic chicken muscle a decreased concentration of acetylcholinesterase in the endplates and concluded that a neural factor which determines the synaptic concentration of this enzyme is defective in this disorder.

The relevance of such findings to the pathogenesis of human muscular dystrophy is uncertain because the diseases in such animal models differ in some important respects from the human muscular dystrophies. McComas and Sica (*Lancet*, **i**, 1119; 1970) and McComas, Sica and Currie (*Nature*, **226**, 1263; 1970), however, described an electrophysiological technique for estimating the number of functioning motor units in the extensor digitorum

brevis muscle of the foot in human patients suffering from the Duchenne, limb girdle and facioscapulohumeral varieties of the disease, and found that the number of functioning motor units was greatly reduced in such cases, even early in the course of the disease. McComas *et al.* suggested that human muscular dystrophy might therefore be caused by a lack of neuronal trophic factor upon which the function and survival of muscle fibres depend.

This concept that muscular dystrophy might well be attributable to a primary neuronal abnormality (the "sick" motor neurone) has become known as the "neurogenic hypothesis". It has been widely challenged, first by histopathologists who have found the earliest pathological changes in human dystrophic muscle to be very different from those of naturally-occurring denervating processes, and by others who have suggested that electrophysiological studies carried out on a muscle (the extensor digitorum brevis) which is not affected early by the dystrophic process, cannot be accepted as being a reliable guide to the nature of the primary aetiological process. Furthermore, Papapetropoulos and Bradley (*J. Neurol. Neurosurg. Psychiat.*, **35**, 60; 1972) have found that even in dystrophic mice the total number and morphology of the spinal cord anterior horn cells are normal.

It is true that Bradley and Jenkinson (*J. Neurol. Sci.*, **18**, 227; 1973) found striking histological abnormalities in the anterior spinal roots of such dystrophic animals, but they gave cogent reasons for suggesting that these neuronal abnormalities are not likely to be of primary aetiological importance. Furthermore, as already mentioned, clinical, electromyographic and histopathological studies in patients previously diagnosed as suffering from limb girdle and facioscapulohumeral muscular dystrophy have shown that many such individuals are suffering from a relatively benign spinal muscular atrophy. It is thus possible that the diagnosis of muscular dystrophy in some of the patients examined by McComas and his colleagues was incorrect, but this criticism seems likely to be invalid in relation to the patients with Duchenne type muscular dystrophy whom they studied, as the latter disease seems still to be a clearly defined and consistent clinical and genetic entity. Thus the role of the motor neurone in the pathogenesis of the muscular dystrophies continues to be a topical and controversial question.

On page 287 of this issue of *Nature*, Kümpel and Dubowitz describe a series of interesting tissue culture experiments in an attempt to clarify the part played by the motor neurone in the pathogenesis of murine muscular dystrophy. They had previously shown (Bishop, Gallup, Skeate and Dubowitz, *J. Neurol. Sci.*, **13**, 333; 1971; and Gallup, Strugalska-Cynowska and Dubowitz, *ibid.*, **17**, 109; 1972) that dystrophic muscle explants obtained from dystrophic mice and from human subjects showed normal growth characteristics *in vitro*, and they were unable to differentiate between normal and dystrophic muscle grown in culture. In the present series of experiments, they cultured muscle cells along with spinal cord explants

taken from mouse embryos and found that regeneration and growth of the muscle cells were stimulated by contact with ventral root neurites emerging from the spinal cord explants. Significant differences, however, were found when they cultured normal nerve with normal muscle, normal nerve with dystrophic muscle, dystrophic nerve with normal muscle, and dystrophic nerve with dystrophic muscle. When contacted by ventral root neurites from normal spinal cord, the muscle cells of both normal and dystrophic muscle fused to form new fibres with well-developed cross striations and peripherally-located nuclei. The appearances in muscle coupled with "dystrophic" spinal cord were quite different. The initial cellular regenerative response was followed in most cases by degeneration and disappearance of all muscle elements, leaving only strands of connective tissue. In only three cultures were myotubes formed, and these failed to develop synchronized contractions.

On the basis of their evidence, Kumpel and Dubowitz have reasonably concluded that murine dystrophic muscle behaves morphologically quite normally when regenerating in the presence of normal spinal cord, and, second, that regeneration of both normal and dystrophic muscle is severely abnormal when coupled with spinal cord explants from dystrophic mice. These findings would certainly support the view that in murine muscular dystrophy at least, the primary abnormality lies in the neurone. Whether further investigations, which must be indirect, in view of the impossibility of obtaining viable human anterior horn cells for culture, will confirm that there is a similar defect in the human disease, is, of course, uncertain. But the field is one of rapid development and new information is eagerly awaited.

From a Correspondent

INFECTIOUS HEPATITIS

From Man to Marmoset

from our Medical Virology Correspondent

THERE have been many attempts to propagate human hepatitis viruses outside human volunteers. The observation in 1961-62 of infectious hepatitis (hepatitis A) among human handlers of potential astronaut chimpanzees, and subsequent reports of more than forty small clusters of cases of viral hepatitis in human beings in close contact with

young monkeys which had been recently imported, revived interest in experimental transmission studies to non-human primates (*Viral Hepatitis: Wld Hlth Org. Tech. Rep. Ser.*, No. 512; 1973). The disease was not, however, transmitted consistently from animal to animal and interpretation was further complicated by spontaneous hepatitis in some monkeys.

Working on the assumption that primates which have little or no contact with man are unlikely to have acquired immunity to the human hepatitis virus, Deinhardt and his colleagues (*J. Exp. Med.*, **125**, 673; 1967) have made use of marmosets, a species of small South American monkey. These monkeys are ferocious, so that contact with man tends to be minimal, and serological surveys of antibody have confirmed that naturally acquired infection to common human viruses is rare. Biochemical and histological changes compatible with hepatitis were found after the inoculation of acute phase serum or plasma from patients with acute viral hepatitis in two species of marmosets, *Saguinus nigricollis* and *S. fuscillois*. Liver damage, without clinical evidence of infection, was produced regularly during five serial transmissions from marmoset to marmoset. Further evidence that marmosets of the species *Saguinus mystax* are susceptible to human hepatitis A, but not to human hepatitis B, was provided by Lorenz *et al.* (*Proc. Soc. Exp. Biol. Med.*, **135**, 348; 1970). Biochemical and histological evidence of liver damage was induced by injecting marmosets with serum derived from a pool of sera obtained from volunteers who were suffering from experimentally transmitted hepatitis A.

Confirmation of these results has just been published. Mascoli *et al.* (*Proc. Soc. Exp. Biol. Med.*, **142**, 276; 1973) inoculated intravenously, during the course of five years, 274 marmosets, chiefly of the species *Saguinus mystax* and a few *S. nigricollis*, with blood from human cases of hepatitis A or hepatitis B. It was found that the indispensable element for the induction of hepatitis in marmosets was the introduction of blood from patients with hepatitis A. As an in-built control, serial passage was carried out in marmosets of a pool of sera obtained before inoculation from marmosets in which the hepatitis A agent was subsequently propagated. Spontaneous hepatitis in marmosets was not encountered.

Blood specimens from five out of seven patients with acute hepatitis A in Costa Rica induced hepatitis in the marmosets. Blood samples taken from patients 22-29 days before the onset of illness yielded negative findings in seventy marmosets. Pools containing samples obtained during the acute illness or during convalescence (29-113

days after the onset of illness) caused hepatitis in 24 of 119 animals. The agent of hepatitis A was passaged successfully four times and it was concluded that the marmoset is highly reliable for detecting, propagating and studying human hepatitis A.

Deinhardt and his colleagues (*Nature*, in the press) have now provided the requisite final piece of evidence by neutralizing the disease in marmosets with serum from convalescing hepatitis patients. The infectivity of the acute phase MS-1 serum from human volunteers and its infectious fractions prepared by density gradient centrifugation in caesium chloride was neutralized with convalescent patient serum, whereas infectivity was unaffected by incubation with human serum albumin or by incubation with preinoculation serum from the same volunteers.

These recent results present exciting advances in the hitherto insoluble problem of the common form of viral hepatitis and the way is now open for investigating the physical and biochemical characteristics of the human hepatitis A virus(es).

FISH STOCKS

Parasites and Whiting

from our Marine Vertebrate Correspondent

THE use of parasites as indicators to supply information on the migrations, past history and even distinctness of stocks of fishes has been a developing and fruitful field of study in recent years. In a new report Shotter (*J. Fish Biol.*, **5**, 185; 1973) applies this technique with conspicuous success to the stocks of whiting (*Odontogadus melanogus*) off the Isle of Man. The biology of this fish is in general well known, but its early life history and especially the relationship of offshore stocks and postulated nursery grounds inshore are not firmly established.

It has long been known that the larvae of the whiting are planktonic until about 15 mm in length, when they adopt a commensal relationship with medusae, living under the shelter of the jelly fish—a stage in their life which seems to be essential to their wellbeing. It has been suggested that at the conclusion of this phase they migrate into inshore nursery areas where they feed until they adopt a benthic mode of life and move offshore when they are around 21 cm long.

Shotter's conclusions cast considerable doubt on the validity of this general sequence. He has studied the parasitic fauna of whiting collected in Port Erin Bay, and from two offshore sites in Manx waters. His study has thrown much light on the parasitic fauna and the incidence of the species at different periods of the host's life. This alone represents a considerable advancement

to knowledge of this fish and its parasites.

It seems that even the commensal relationship with jelly fishes is not without parasitic hazards for the young whiting. Of the forty-one young fish (15–42 mm in length), thirty were hosts to the copepodids of *Caligus*, which it is believed is itself attracted to the shadow of the medusal umbrella. After about 3 months, however, the association with the medusae ceases and the infestation with *Caligus* declines markedly.

Shotter's main intention was to demonstrate any differences between parasites of populations in different areas and where possible to relate them to feeding habits, physical characteristics of the differing environments and migrations. The comparisons possible between the parasites found in inshore and offshore whiting populations are striking. To cite but two of the ten species of internal parasite listed, the monogenean *Diclidophora merlangi* was found in 62% of the offshore fish but in only half this number of the inshore population, and the digenean *Podocotyle atomon* occurred in only 0.8% of the former, but in 47% of the inshore fishes. Another digenean, *Cryptocotyle lingua*, occurred as metacercariae in 28% of the offshore fish but in 67% of those found in inshore waters.

Shotter relates quite convincingly the incidence of these parasites to the local environment of the fish and its food. Thus *Diclidophora* is a direct parasite without intermediate hosts. From the age of about 6 months, the whiting adopts a benthic mode of life and becomes susceptible to infestation from the bottom-living early stages of the monogenean. Large infested whiting are found offshore so the continuance of the life cycle is ensured. *Podocotyle*, on the other hand, has as its first host certain mysid and amphipod crustaceans which are an important food only for the inshore fish, and this parasite is characteristic of fishes which have spent a long time in inshore waters. Similarly *Cryptocotyle* has a life cycle involving the shore-dwelling gastropod *Littorina littorea*, a fish, and as final host one of several species of sea gull; it too is typical of inshore fishes.

The conclusion that Shotter draws from his study of the whiting's parasites is that any migration from the postulated inshore nursery areas to deeper water is probably restricted to a few individual fishes. He suggests that the majority of the inshore fishes may die prematurely as a result of their heavy burden of parasites and increased predation, and that two years is probably the maximal life span of an inshore whiting. It seems from his work that offshore stocks are not recruited from inshore nursery areas as had been thought, a result of some significance in a species which is heavily exploited by the fishing industry.

OIL POLLUTION

Recovery of Salt Marsh

from our Plant Ecology Correspondent

IN spite of the abundance of dogmatic assertions regarding the effects of pollution on the environment, which emanate from polluter and conservationist alike, there are surprisingly few experimental or observational data on changes in natural communities which can be directly related to pollution. This applies particularly to plant communities. Where experiments have been conducted, or observations made, they have usually been concerned with the effects of acute, high intensity pollution rather than with those of long-term, low-level chronic pollution.

The Field Studies Council Oil Pollution Research Unit, based at Orierton, Pembrokeshire, has been concerned with the long-term effects of successive oil spillages on maritime communities. It is known that shore communities recover well from single spillages, as is evidenced by the case history of the Torrey Canyon. At Milford Haven, where there is a large oil port, small, successive spillages of oil present a recurrent problem and their long-term effects are unknown.

In a recent report (*Environ. Pollut.*, 4, 223; 1973), Baker describes the results of four years' observation on experimentally polluted areas of salt marsh in the Milford Haven area. In 1968 three areas of salt marsh which represented successional stages in salt marsh development were selected — low level *Spartina* marsh, mid level *Puccinellia* marsh and high level *Juncus maritimus* marsh. A block of randomized 2 m × 5 m plots was set up in each of the three

areas, one being a control, untreated plot and the others receiving monthly sprayings with Kuwait crude oil. Treatments consisted of two, four, eight, or twelve successive monthly sprayings. Changes in vegetation were recorded by means of point quadrats or, in the case of *Spartina*, tiller densities.

The effects of these treatments varied in the different communities. Four or more oilings produced significant reductions in *Spartina* density in the following year. All treatments had recovered within four years, but this was largely because of reinvasion of the plots from surrounding healthy colonies. Successive spillage on a wide scale would probably have a longer-lasting effect: eight or more sprayings in the mid-level salt marsh resulted in a spectacular reduction in the grass *Puccinellia*, which has shown no sign of subsequent recovery. Similar effects were observed in the high-level marsh for *Festuca rubra* and the sea rush, *Juncus maritimus*. In the upper marsh the grass *Agrostis stolonifera* has taken advantage of the niches left vacant by the elimination of these species and has increased considerably.

These data will be of considerable value in predicting the effects of oil spillage on salt marshes. The smaller diversity of vegetation which results from such treatment could lead to lost stability, erosion and the redistribution of large quantities of mud. It would be interesting to know, however, whether the profound effects of eight or more sprayings would have resulted if the treatments had been more widely spread over the four-year period, rather than being concentrated into the first year. It may be that a critical recovery period of more than one month is required for the maintenance of vegetational stability.

DNA Conformation and Initiation of Transcription

WHEN the transcription of DNA by RNA polymerase is initiated the enzyme must bind specifically to the DNA template. Obviously therefore there are two ways in which this initiation step may be regulated, either by changing the structure of the polymerase or by changing the structure of the DNA template. The role of factors such as sigma factor which associate with the polymerase and affect the specificity of initiation has been partially elucidated, but less attention has been given to the effects of changes in conformation of the template DNA on initiation. Travers, Baillie and Pedersen have, however, been investigating this question by measuring the rate of synthesis *in vitro* of *Escherichia coli* ribosomal RNA by *E. coli* RNA polymerase in various conditions which alter the configuration of the DNA template.

As they report in *Nature New Biology* next Wednesday (June 6), using a standard reaction mixture they noticed

a lag in the rate of ribosomal RNA synthesis. By varying the order of addition of the components they quickly showed that the DNA template had to be "activated" before efficient transcription began; moreover this activation step seemed reversible. By varying the salt concentration in the incubation mixture, as well as the temperature of incubation and using T4 DNA as a template in some experiments, Travers and his colleagues reached the conclusion that "the substantial changes in both total and rRNA syntheses over a narrow temperature range bear a striking resemblance to a nucleic acid melting curve and suggest that cooperative structural changes in the DNA template may occur". If these changes occur at promoter regions they would, of course, influence the pattern of transcription. In short the activity of a promoter may be controlled directly by controlling its conformation.

PLANT CONSERVATION

Flora Macaronesia

from a Correspondent

THE term Macaronesia is a collective name for the Atlantic Islands including Madeira, the Azores, the Canary Islands and the Cape Verde Islands which have long been recognized by plant geographers as together forming a floristic region characterized by many common elements associated with high endemism; possibly 700 species of flowering plants are endemic to the region.

Unfortunately many of these unique species are now gravely endangered—some are now represented by only a few individuals in a single locality; some may now be extinct. For scientific and historical reasons, their conservation and study have become urgent. Both aspects were discussed in detail at the first international congress "pro Flora Macaronesia" held at Las Palmas, Gran Canaria, in April. Some seventy-two botanists from the Canary Islands, Denmark, France, Germany, Great Britain, Italy, Jordan, Madeira, the Netherlands, Norway, Portugal, Spain, Sweden and the United States participated and provided convincing evidence of the increasing international interest in the Macaronesian flora.

The most important of the decisions made by the congress concerned the preparation of a *Flora Macaronesiaca*, which would provide a synoptic account of the plants of the region, and proposals for the conservation of areas of high scientific importance because the delegates sadly but realistically were well aware that complete conservation is now impossible against the growing demands of tourism, population growth and land speculation, particularly in the Canary Islands. Every island has its own peculiar specialties. A menace to the survival of many endemic species hitherto sheltered by their isolation is the harsh competition of many vigorous introduced alien plants, frequently introduced as ornamental or economic plants and now exterminating the native vegetation.

BIOMATERIALS

Prostheses and Tissue

from a Correspondent

AT the fifth annual biomaterials symposium organized by Clemson University, South Carolina, on April 14, the theme for discussion was prostheses and tissue: the interface problem.

Participants critically scrutinized some of the devices, including buried dental implants and knee and hip joint replacements made from stainless steel, cobalt-chromium alloys and titanium, which are in clinical use on a large scale

at present. Numerous patients suffering pain and disability have obtained very considerable short term benefit from these implants, but there is disquiet about the long term results. This point of view was summarized by Dr C. Hirsch (Karolinska Institute, Stockholm) who, in reviewing clinical problems in total hip replacements, said, "Although it is probably an achievement to be able to implant joints, it is obvious that we are far from a clinical situation that can be tolerated".

One clinical problem is infection; another is loosening of the implant with time. The metallic or plastic components used to replace diseased joints are usually fitted to the skeleton with the aid of acrylic resin which is mixed in the operating theatre and allowed to polymerize *in situ*. Acrylic cement has made this type of surgery possible on a large scale, but it is widely recognized that the method is not ideal. Cold-curing acrylic is comparatively weak and brittle and some unchanged monomer, which is toxic, may be present at the end of the polymerization reaction. Patients on the operating table some-

times suffer a sudden drop in arterial pO_2 and cardiac arrest as was discussed by Dr P. I. Kenmore (George Washington University), who recommended that patients should be well oxygenated just prior to and for 15 minutes after the insertion of the acrylic bone cement. The polymerization reaction is exothermic and Dr E. P. Lautenschlager (Northwestern University, Chicago) has measured temperatures up to 70° C at the acrylic-bone interface. Thermal damage and necrosis of tissues, the poor flow and conforming qualities of acrylic dough and its inherent weakness may be why prostheses sometimes work loose.

A third problem has to do with the tissue reaction to minute particles of metallic alloy and their corrosion. Drs D. F. Williams (University of Liverpool) and G. D. Winter (Institute of Orthopaedics, Stanmore) both reported that there are three types of foreign-body particles commonly found in tissues around stainless steel joints and corroded implants: (1) particles of the steel alloy; (2) platy deposits, thought to be corrosion products, which cause a giant cell

Histone Phosphorylation and Chromosome Structure

A ROLE for histones in the control of genetic activity has been proposed for many years. But histones fell out of favour as control molecules when it was found that their number (and therefore versatility) was small and that their amino acid composition was almost identical in all tissues and, furthermore, in species as far removed as the pea and the cow. More recently, however, interest in histones has been revived with the discovery that these proteins can be enzymatically modified by phosphorylation and acetylation. In *Nature New Biology* next Wednesday (June 6), Louie and Dixon describe studies on the kinetics of phosphorylation and dephosphorylation of testis histones which suggest that such histone modification plays a part in determining chromosome structure.

The kinetics of phosphorylation and dephosphorylation of histone I in trout testis suggest that both "old" and "new" histone I undergo extensive phosphorylation and dephosphorylation throughout the cell cycle. Histones IIb₁ and IV are also extensively phosphorylated, but the kinetics differ from those of histone I. These kinetic differences indicate that specific phosphokinases and phosphatases exist in the cell for each principal histone fraction. Because the half-life of ³²P-labelled histone phosphate is 6–8 h, the maintenance of high levels of phosphorylated histones I and IV in trout testis cells over long periods suggests that enzymatic modification of

these histones is a continuous balanced process.

Louie and Dixon have proposed previously that the phosphorylation of histones IIb₁ and IV is concerned with the correct binding of these histones to chromatin. They now suggest that the extent of phosphorylation of histone I molecules in a particular stretch of chromatin may determine the degree of supercoiling of the chromosomal fibrils. Fibrils in which histone I is completely phosphorylated would be in the extended form (30–40 Å diameter), intermediate degrees of phosphorylation would allow the extended form to collapse into 100 Å supercoils, and still lower levels of phosphorylation might give rise to coiled supercoils of 200–300 Å diameter. Louie and Dixon have calculated, from their data, the molar phosphate to histone I ratio in the 35 Å, 100 Å and 200–300 Å fibrils and have found that the figures agree well with experimentally determined levels of phosphorylation of histone I in euchromatin and heterochromatin.

The continuous phosphorylation and dephosphorylation of histones I and IV involved in the persistence of the histone in a phosphorylated state suggest that the maintenance of the euchromatic state is an active metabolic process. Regulation of the order of coiling of chromatin fibres by phosphokinases and phosphatases might constitute a coarse control of DNA replication and transcription.

reaction and are fibrotic and necrotizing; and (3) haemosiderin-like granules which have been identified as a mixture of iron oxides and hydroxides.

Titanium has been positively identified at levels in excess of 2,000 p.p.m. in the tissues around some titanium implants. Particles of cobalt-chromium and carbides from the alloy are found in macrophages near cobalt-chromium prostheses. A granulomatous reaction associated with very minute particles near cobalt-chromium total joint replacements was described. The severity of the local tissue reaction depends on the size, shape, chemical nature and particularly on the number of particles present. The otherwise unexplained pain which some patients with prostheses suffer may be related to these foreign-body tissue reactions.

Research is being pursued into the fabrication of materials into which bone will grow and lock the implant into the skeleton. Porous ceramics, titanium, polythene and various forms of pyrolytic carbon are being investigated. Special techniques which have been developed for the histological study of these hard materials were described by Dr J. J. Klawitter (Clemson University).

Dr S. F. Hulbert (Clemson University) and his colleagues have established that the minimum size of interconnecting pores for the ingrowth of bone into calcium aluminate ceramic pellets is 40–100 μm and the material appears to be well tolerated by the tissues. Dr G. D. Winter said that 50 μm was the critical pore size for the induction of heterotopic bone in certain synthetic sponges in the skin of the pig.

Dr L. L. Hench (University of Florida) demonstrated that a cementoid layer forms between surface active glass and glass-ceramic implants and healing bone bonding the two together. The glass surface is purposefully formulated to release sodium, calcium, phosphate and silicon ions. Collagen fibres become incorporated within the 600–1,000 Å cementoid layer and mineralization occurs. In rats, after 28 weeks, the bone to implant bond is stronger in torsion than the bone itself.

A recent report of the clinical use of induced electrical fields to heal a congenital pseudarthrosis of the tibia has stimulated renewed interest in electrical phenomena in tissues. With commendable enthusiasm Dr R. O. Becker (Veterans Administration Hospital, Syracuse) sees the electrical stimulation of hard tissue growth as an alternative to prosthetic devices and he reported success with rabbits in obtaining new growth of articular cartilage over defects on the femoral condyle by implanting bimetallic electrogenic devices.

Next year's symposium will be about the selection of materials for reconstructive surgery.

FEBS

New Foods

from a Correspondent

At the special meeting of the Federation of European Biochemistry Societies (FEBS) in Dublin between April 15 and 19 (see also this week's issue of *Nature New Biology*, **243**, 130; 1973), one of the most interesting contributions was the description by Drs J. F. Connolly (The Agricultural Institute, Dublin), M. Noonan and Professor M. G. Harrington (University College, Dublin) of a physiological mechanism for producing a polyunsaturated lamb chop.

In the body fat of all ruminants (for example, cows and sheep) there is a relatively high concentration of saturated fatty acids which result in part from the hydrogenation of unsaturated fatty acids by microorganisms in the rumen. By bypassing the rumen it might be possible to avoid hydrogenation and produce special products suitable for patients being treated for coronary heart disease — containing polyunsaturated fatty acids. Indeed in 1961 Ogilvie *et al.* (*Nature*, **190**, 725) avoided the rumen by administering fatty acids directly into the abomasum (or small intestine), and in 1970 a similar result was obtained in Australia by Cook *et al.* (*Nature*, **228**, 178) who fed cows with specially coated seeds as dietary supplements that were rich in polyunsaturates. It was thus possible to produce milk with a high level of such fats and it has been

shown that diets containing these products can lower the blood cholesterol.

Dr Connolly and his colleagues exploited a physiological mechanism (the oesophageal groove reflex feeding system (see Ørskov and Benzie, *Brit. J. Nutr.*, **23**, 415; and Lawlor *et al.*, *loc. cit.*, **26**, 439; 1971) by which the suckling ruminant automatically passes food beyond the rumen) to feed lambs substitute diets rich in polyunsaturated fatty acids. A special milk substitute high in linoleic acid was used. The lambs were killed at 36 kg and analyses showed that the body fats of the animals fed the special diet contained ten times as much polyunsaturated fatty acid as the control lambs. Muscle lipids showed a similar trend. It is not without interest that cutlets prepared from these "polyunsaturated lambs" were indistinguishable from those of lambs fed in the orthodox way. The availability of such lambs is commercially feasible so that it should be possible now to formulate special diets aimed at lowering the levels of blood lipids in man. At present only a very limited range of natural appetizing foods are available to patients on cholesterol-reducing diets. Dr Connolly and his colleagues have now begun experiments to produce a veal type calf using a similar feeding system.

Turning from fats to proteins, Professor A. Spicer (The Lord Rank Research Centre, High Wycombe) may well have raised the price of beef in Dublin with his well founded prediction "no beef by 2000". The trouble was that cattle are

New Variant for the Haemoglobin Collection

An interesting new abnormal haemoglobin, with a functional defect, is described by Bromberg *et al.* in *Nature New Biology* next Wednesday (June 6). It has been termed haemoglobin Little Rock (LR), and is unique among the variants so far studied in that it has an anomalously high oxygen affinity without any accompanying diminution in the haem-haem interaction, or any change in the Bohr effect.

A fingerprint analysis establishes that the substitution is glutamine for histidine at position 143 in the β -chains. This is known from X-ray work to be part of the binding site for the cofactor, 2,3-diphosphoglycerate. In the haemolysate the oxygen affinity of haemoglobin LR is greater by a factor of three than that of normal haemoglobin in the adult human.

This difference is not eliminated when the cofactor is stripped off, although the effect of diphosphoglycerate in displacing the oxygen binding curve is appreciably lower than in the normal protein. Evidently, therefore, the affinity for the cofactor is decreased as a result of the substitution by a factor estimated as about 2.5. This effect cannot, however,

explain the difference in oxygen affinity.

In an accompanying note, Perutz reports on the consequences of the substitution in terms of the molecular model of haemoglobin. The normal his-143 is not engaged in any interaction with other side chains in either the oxy or the deoxy form, though it does make contact with the cofactor when this is present. A glutamine in the same position in the oxy form is so placed as to make a hydrogen bond with asn-139 in the other β -chain. On deoxygenation, the relative displacement of the β -chains would lead to rupture of these bonds. Thus the oxy-structure in haemoglobin LR is stabilized by the extra hydrogen bonds, and in addition the interaction with the diphosphoglycerate in the deoxy form is weakened because a salt bridge is missing in either β -chain.

Perutz predicts on the basis of these inferences that the dissociation of tetramer to dimer should be inhibited in the liganded forms of haemoglobin LR, and that in the absence of cofactor the increased oxygen affinity should be associated chiefly with a change in the association constant, K_4 , for attachment of the last oxygen to the tetramer.

extraordinarily inefficient converters of crop products into protein. Whereas the cow puts on only 1 pound of protein a day, pound for pound growing soya beans put on 100 pounds a day, yeasts put on 100,000 pounds a day and bacteria put on 10^{14} pounds a day.

Dr F. Moran (British Petroleum, Surrey) elucidated the possibility of producing protein foodstuffs from petroleum byproducts. One of the plants run by British Petroleum is turning out 17,000 tons of this food a year, and future plants will have an annual capacity of 100,000 tons. The protein in question has been found to be nutritious and safe and Dr Moran said that it has been cleared as animal foodstuffs in France, Italy, Great Britain, Germany, Denmark and Holland. Clinical tests for human foods were being done and their possible use as additives in baby foods or confectionery was being considered.

On the subject of pollution Dr C. F. Seyfried (Technical University, Hanover) made the prediction that the technique of reverse osmosis would play a very important part in the future treatment of effluents and Dr I. Tookos (Research Institute for Water Resources Development, Budapest), in her presentation on the treatment of organic effluents from the food industry, was quick to point out that biological degradation was preferable.

Dr H. J. Hueck (Central Laboratory TNO, Delft) predicted that the future use of mercury will be severely restricted, and he discussed in particular the use of protective materials containing copper and mercury. "The River Rhine," he said, "carried some 2,900 tons of copper and 70 tons of mercury each year, while Dutch industry—such as metal plating and paint production—spews forth annually 500 tons of copper and 46 tons of mercury."

PROTEINS

Micromethods

from our Molecular Biology Correspondent
THOSE horny-handed toilers of biochemistry who have spent the greater part of their adult life in coaxing tiny cleaning wires through blocked holes in recorder pens, or other occupations of a similar order, might well break out in a craven sweat at the very idea of attempting analytical protein chemistry on extracts from single cells. Nevertheless, gel electrophoresis techniques have, for example, been developed to cope with quantities of protein in the nanogram range, or even below.

A variant that has now been described is isoelectric focusing, done in acrylamide gels of 1 mm or so diameter. Gainer (*Anal. Biochem.*, **51**, 646; 1973) has found it possible to fractionate proteins

by this means, and to determine the isoelectric point of a 10^{-10} g sample. As in the usual gel electrofocusing technique, the polyampholyte, which is responsible for establishing the pH gradient, is included in the polymerization mixture. The separation, fixing and staining follow more or less the standard large-scale procedures, with certain variations, the trickiest manipulations being those concerned with application of the sample. Results with standard proteins show that the micromethod yields little in the precision of the measured isoelectric points to the standard procedure, using a thousand times more protein. Gainer has already applied the technique to the examination of proteins extracted from single neurones.

The sensitivity of fluorescence, especially of extraneous chromophores chosen for their high quantum yield and capable of attachment to proteins and amino acids, has likewise been put to good use for a number of microanalytical purposes, including, for example, end-group determination. An interesting discovery is now reported by Kinoshita, Iinuma and Tsuji (*Biochem. Biophys. Res. Commun.*, **51**, 666; 1973), who found that the cyclic oligosaccharide,

cycloheptaamylose, will sequester dansylamino acids in aqueous solutions, with a huge increase in the fluorescent intensity from the dansyl group. The enhancement is substantially insensitive to added solutes, other than those which themselves form complexes with the saccharide. The sensitivity of the fluorescent assay is such that concentrations of the amino acids after dansylation can be determined at levels well below nanomolar. Kinoshita *et al.* have examined the interaction of the dansyl derivatives of all the common amino acids with cycloheptaamylose, and found that all form 1:1 complexes with rather similar free energies, but that the enthalpic and entropic contributions vary somewhat. The small, or unfavourable, entropy changes found for the hydrophobic amino acids can in principle be explained by solvation effects.

Another fluorometric assay for proteins, peptides and amino acids has been evolved by Udenfriend and his colleagues, who discovered that the ninhydrin reaction of primary amines could be modified by the inclusion of phenylacetaldehyde in the reagent, so as to generate a new and blindingly fluorescent chromophore. Since then the nature of this species has been established, and a

Global Tectonics in the Precambrian

DURING the past few years geologists have succeeded in establishing some of the types of geological structure formed early in the history of the Earth. It is, however, far from certain how these structures are related to one another and still less certain what systems of global tectonics operated early in the Precambrian. The successions of volcanic rocks preserved in the Precambrian greenstone belts have no exact analogue today; in some respects they resemble the volcanic materials which accumulate on island arcs but in other respects the rocks can be compared with the predominantly basaltic successions erupted along modern ocean ridges.

Particular attention has been paid to Precambrian anorthosites and it is now clear that in addition to the well known late Precambrian examples, anorthosites were formed rather extensively much earlier in the Precambrian. Two belts in particular have been identified; one extended from North America through Greenland into north-west Europe; the other ranged from Central Africa through Madagascar into India.

Although the exact ages of the anorthosites in these belts are still imprecisely known, they seem to date from a period between 3,500 and 2,500 million years ago. It is known that granitic rocks had already evolved by that period, and this has led to the suggestion that the granitic rocks may represent early continental crust and the anorthositic associations

might represent a type of Precambrian oceanic crust. Garson and Livingstone (see *Nature Physical Science* next Monday, June 4) apply this hypothesis to a small area rich in anorthosites which is exposed in South Harris off north-west Scotland; they suggest that the Scottish anorthosites and the associated rocks might represent a piece of oceanic crust thrust into juxtaposition with fragments of a Precambrian continent.

The areas available for study in the Outer Hebrides are so small that it is likely to prove very difficult to arrive at a reasonable interpretation of the large scale structural relations, but it may be possible to do this in Greenland or in the Canadian Shield where more extensive outcrops are present. In this connexion it is interesting to recall that McGregor reported recently that he and colleagues from the Greenland Geological Survey with experience in Western Greenland had arrived at a hypothesis which interpreted the anorthosite rich Precambrian regions in Greenland as primitive oceanic crust.

It will be of interest to see if further work confirms the suggestion arrived at independently by these two groups of workers, and whether future research can establish the relationships of the anorthosites to the greenstone belts and show whether or not these Precambrian igneous associations resemble in any way successions of igneous rocks erupted at the present day.

reactive derivative synthesized. This substance, called fluorescamine, is itself barely fluorescent, reacts more or less instantaneously with amino groups to yield the fluorescent product, and can therefore be used as the basis of an assay (Böhlen *et al.*, *Arch. Biochem.*, **155**, 213; 1973). The fluorescent response is linear with concentration, and the sensitivity is limited by the presence of traces of primary amines as impurities. Under these circumstances the method can be used to estimate 500 ng and up of protein. If, however, impurities of low molecular weight are stripped out by gel filtration in an automated apparatus, this lower level falls by at least an order of magnitude. The response depends of course on the lysine content of the protein, so that for an accurate determination a calibration with the same protein is necessary, but this drawback is, as Böhlen *et al.* point out, no greater than that of the Folin reaction and other colorimetric methods.

In an accompanying report from the same laboratory, Stein *et al.* (*ibid.*, 203) describe the most spectacular application of fluorescamine. If it is used in place of ninhydrin in an amino acid analyser, and the products are monitored by fluorescence instead of absorption, then the sensitivity begins to approach the picomolar range, that is to say 1 μg of protein suffices for amino acid analyses of the highest quality. Hydrolysis is performed in the usual way, and derivatization of the amino acids follows their separation on the ion-exchange columns. Very narrow columns can then be used, with correspondingly greater flow rates, less diffusion, and thus better separation, for a given pumping speed. The reaction is practically instantaneous, and does not need heating.

The possibilities that the method now opens should exercise the minds of many biochemists. Stein *et al.* have already found it possible to determine free amino acids in microlitre volumes of plasma and to perform analyses on minute quantities of proteins in slices of stained polyacrylamide gels, because the ammonia generated during the hydrolysis of the polyacrylamide does not interfere with the fluorescamine reaction, or itself give rise to a highly fluorescent product. A commercial version of the fluorescence-based amino acid analyser is in prospect.

LIQUIDS

Centenary Remembered

from a Correspondent

THE third of a series of conferences on the liquid state, started by the Institute of Physics and Physical Society in 1963, was held at the University of Kent from April 16 to 18, and was on the work of van der Waals and the relationships of

this work with contemporary and later developments.

Professor J. S. Rowlinson (Imperial College, London) opened the programme with a paper on the legacy of van der Waals which is to be published in *Nature* in due course. Professor Rowlinson gave a careful account of the history of van der Waals's work on the equation of state, on the critical region and on mixtures, and showed how far it amounted to a synthesis of already existing ideas without detracting in any way from its greatness. Several later speakers returned to this theme of "history repeats itself". For example, Drs A. J. B. Cruikshank and J. W. Goodwin (University of Bristol) showed how van der Waals's ideas are still helpful in interpreting residual functions of liquid mixtures, and Drs Y. de Ribaupierre and F. D. Manchester (University of Toronto) suggested that the hydrogen-palladium system can usefully be compared with a van der Waals gas, the distension of the palladium lattice acting rather like a long-range attraction between the hydrogens.

One of the principal themes was the interplay of different methods. For example Professor J. E. Enderby and his colleagues (University of Leicester) described a comparison of X-ray and neutron studies of strong aqueous solutions of NiCl_2 , the scattering power of the nickel for neutrons being changed by isotope substitution. Some very interesting conclusions were drawn about this complicated system. Also of interest is the fact that considerable information is obtainable from light scattering (Professor G. Benedek (MIT)) and ultrasonics (Professor D. Sette (University of Rome)) in spite of the fact that the wavelengths are much greater than atomic spacings.

It was also of interest to hear about the state of the art of computer simula-

tion of liquid-like systems, for example, diatomic liquids, fused salts, hard non-spherical molecules, which could certainly not have been tackled until quite recently. Highlights were the invited paper by Dr A. Rahman (Argonne National Laboratory) showing what could be done with only thirty-two "molecules", and the film by Dr R. W. Hockney *et al.* (University of Reading) showing strange "collective" movements in an ionic liquid near its solidification point.

One of the growth points of theory is the study of models of polar liquids. Professor G. Rushbrooke (University of Newcastle upon Tyne) showed how perturbation theory, introducing dipolar interactions into a reference system (for example, the hard sphere fluid), can now be carried through systematically. Dr O. R. McDonald (Royal Holloway College, London) showed the good accuracy now obtainable. Agreement with Monte Carlo calculations is found to be very good except in the notorious region near the triple point which always proves difficult to handle theoretically.

Perhaps the most interesting contribution was the last in the programme. Professor Benedek gave several fascinating examples of the application of optical methods to biological problems. It is now possible, for example, to compare the overall size of a ribosome when it is resting and when it is in the process of synthesizing protein. Laser optics has now attained such refinement that the flow velocity of blood in retinal arteries (about 1 cm s^{-1}) can now be measured by Doppler shift in the frequency of the scattered light.

This most enjoyable conference highlighted the advances that have been made since 1963. There is now a good understanding of "simple" liquids which provides a sound foundation for treating more complicated systems.

Radio Source Associated with OH Source ON-1

WINNBERG and colleagues are presently carrying out a search for compact radio sources close to class 1 OH sources, using the Westerbork Synthesis Radio Telescope and the 100 m at Effelsberg. Their search has proved successful with the first object examined, and in next Monday's *Nature Physical Science* (June 4) they describe observations of a source near ON-1.

The radio source is at

$$\alpha = 20 \text{ h } 08 \text{ min } 09.8 \pm 0.1 \text{ s} \\ \delta = 31^\circ 22' 41'' \pm 1''$$

and the OH source position is

$$\alpha = 20 \text{ h } 08 \text{ min } 09.8 \pm 0.5 \text{ s} \\ \delta = 31^\circ 22' 41'' \pm 15''$$

which, as Winnberg *et al.* point out, seems to suggest that the quoted OH position (from studies at CalTech) is

more accurate than the listed errors would imply.

The radio source was unresolved at 5 GHz (WSRT observations) but revealed as a point source at 10.7 GHz (Effelsberg data). Comparison of flux densities at the two frequencies suggests a thermal spectrum with considerable opacity even at the higher frequency. Although only an idealized model can be presented at this stage, the source may, it seems, represent the first stage in the evolution of an H II region.

Most class 1 OH sources are found near large, evolved H II regions because that is where radio astronomers have searched. But it now seems that surveys should be made taking no account of the presence of continuum sources, and that the known sample may be heavily biased by this selection effect.

SELENOLOGY

Lunar Conductivity

from our Geomagnetism Correspondent

IN principle, it is possible to determine the lunar electrical conductivity by measuring the electromagnetic response of the Moon to variations in the magnetic field of the solar wind. In the indirect method, the first step is to calculate the response of a theoretical conductivity distribution and compare it with the response obtained experimentally from magnetometer records. The conductivity structure is then adjusted until the observed and calculated responses agree as closely as possible. Using this technique with data from a magnetometer on the lunar surface and a second magnetometer on the lunar satellite Explorer 35, Sonett *et al.* (*Nature*, **230**, 359; 1971) first obtained an irregular conductivity-depth distribution, the chief features of which were a relatively low surface conductivity and a highly conducting zone at a depth of about 240 km. But Kuckes (*Nature*, **232**, 249; 1971), using the same data, came up with a quite different distribution comprising a uniformly conducting inner sphere and a non-conducting outer shell about 160 km thick. Yet both models agreed with the experimental data to within about one standard deviation.

As Hobbs (*Earth Planet. Sci. Lett.*, **17**, 380; 1973) now points out, if at least two quite different conductivity models can be consistent with the same data, it is reasonable to ask what any model derived from the data means. Accordingly, he has carried out an analysis of the lunar conductivity problem in the light of recent work by Backus and Gilbert (see, for example, *Phil. Trans. Roy. Soc.*, **A266**, 123; 1970) in which the general question of determining infinite-dimensional models from a finite quantity of data is examined. In the case of the Moon, the infinite-dimensional model is the lunar electrical conductivity at all radii, and the finite data are provided by the measured electromagnetic response at various frequencies and the associated estimates of error. Similar considerations would also apply to the Earth, of course; and because data on the response of the Earth to external magnetic field variations are far more abundant than those relating to the Moon, it is instructive at the outset to note the result of applying the Backus-Gilbert ideas to the problem of terrestrial conductivity. As Parker (*Geophys. J.*, **22**, 121; 1971) has shown, the fact is that in spite of the relatively abundant terrestrial data, the conductivity distribution in the Earth is still poorly defined. This emphasizes the importance of determining just what information the more fragmentary lunar

data can give before non-unique conductivity models come to be accepted uncritically as the basis of other investigations.

The specific aim of Hobbs in his first-order analysis was to determine the fineness of detail that can be resolved in the lunar conductivity profile using the available experimental response data. To set a limit on this fineness of detail, he assumed that the data are error free; in other words, if any errors are admitted, the uncertainty in the derived conductivity will be increased. The end product of the analysis is then a smoothed conductivity-depth distribution for each model originally found to be consistent with the data, the models examined being those of Sonett *et al.* and Kuckes.

When the smoothed conductivity profile from Sonett's irregular distribution is compared with that from Kuckes's two-layer distribution, both profiles are found to exhibit little variation in conductivity over the approximate depth range 170–700 km (no estimates are possible below 700 km because there is no field penetration for the frequencies considered) but a lower conductivity in the upper 170 km. In other words, the finest detail resolvable is that of a two-layer model in which the surface layer has a lower conductivity; and the irregularities in the Sonett distribution,

such as the high conductivity zone at 240 km, represent greater detail than the data can sustain. This is not to say that such irregularities do not exist, but only that they are not derivable from a first-order analysis of the available data. Moreover, nor are the smoothed profiles necessarily the most probable or best-fitting distributions; they are merely the smoothest distributions consistent with the data.

The conclusion is, therefore, that in spite of their obvious differences, the models of Kuckes and Sonett *et al.* really only give the same information about the distribution of the Moon's gross conductivity. In view of the fact that both models are based on the same data, this is hardly surprising. But what Hobbs does consider "a little surprising" is the persistence of models containing a thin, highly conducting layer near the Moon's surface. As he has shown, such models are consistent with the data but not derivable uniquely from them; they are the product of inadequate data rather than evidence for the idea of "frescons". Hobbs is thus, in effect, warning against taking the irregular distributions too seriously at this stage and strongly implies that, for example, the lunar temperature profile recently inferred by Duba *et al.* (*Earth Planet. Sci. Lett.*, **15**, 301; 1972) on the basis of the Sonett model is premature.

Sulphur Content of the Earth's Core

EVER since it was discovered that the density of the Earth's outer core is apparently 8–10% lower than the density of pure iron, there has been considerable discussion about the nature of the light component alloying with the iron. Perhaps the most popular contender is silicon, although another possibility—and one that has been receiving increasing attention in recent years—is sulphur. Because of the interest in sulphur, King and Ahrens have carried out a series of shock wave experiments on pyrrhotite to determine whether the equation of state is consistent with the seismic data and (in connexion with a forthcoming report by Usselman) whether the sulphur content inferred is in agreement with the Fe-FeS eutectic composition at core pressures. The results of these experiments are reported in *Nature Physical Science* (June 4) next Monday.

King and Ahrens have obtained shock pressure-density data over the pressure range 198–405 kbar using six pyrrhotite ($\text{Fe}_{0.9}\text{S}$) samples machined from a single crystal, and find that the slope of the resulting Hugoniot is significantly greater than that to be expected on the basis of the ultrasonic data. They conclude from this that the pyrrhotite in the pressure range 289–405 kbar is in a

shock-induced high pressure phase and that at the lowest pressure considered the pyrrhotite is of mixed phase, being only partially transformed to the high pressure phase. The estimated zero-pressure density of the new high pressure phase lies in the range 5.2–5.6 g cm⁻³, which compares with the 4.6 g cm⁻³ of the original material.

By extrapolating the data for the high pressure pyrrhotite phase to the pressure and likely temperature (about 3,500° C) in the outer core (which assumes, of course, that no further phase changes occur) and comparing the result with corresponding pure iron data, King and Ahrens estimate that the core density at the core-mantle interface (9.94 g cm⁻³) is achieved by the presence of 26–31% of the high pressure $\text{Fe}_{0.9}\text{S}$ phase or 10–12% (weight) of sulphur. Further extrapolation throughout the outer core introduces greater uncertainties but, taken at face value, implies a sulphur content of 10–15% to be consistent with the observed profile of pressure-density data.

These new estimates of the supposed sulphur content of the outer core agree well with the values of 10%, 15% and about 9–12% obtained by other workers using different methods.

Tree-ring Calibration of Radiocarbon Dates and the Chronology of Ancient Egypt

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The historical calendar of Ancient Egypt is the only independent chronology for testing the bristlecone pine calibration of the radiocarbon time scale from 3000 to 1800 BC. Here a statistical approach is used to compare the functions relating the Egyptian historical dates to the corresponding radiocarbon dates and the bristlecone pine tree-ring dates to the corresponding radiocarbon dates, and it is concluded that the calibrated radiocarbon dates for Egypt do not differ significantly from the historical dates in the time period considered, although the errors associated with these preclude a precise comparison. The accord gives qualified support for both systems and invalidates some published objections to the use of a calibrated time scale in prehistoric archaeology.

THREE radiocarbon laboratories, at La Jolla, Philadelphia and Tucson¹⁻³, have obtained radiocarbon dates over the past decade for specimens of bristlecone pine already dated dendrochronologically⁴, thereby allowing the "correction" of the radiocarbon scale. The calibration curve produced by Suess⁵ is now widely used for this purpose. Its precise form has been questioned by some workers⁶, especially the validity of the "kinks" or short-term fluctuations in the curve, and the nature of these short-term fluctuations in the atmospheric concentration of radiocarbon remains a problem⁷. In some quarters doubt has been expressed over the reality and magnitude of the first order deviation itself⁸.

The historical calendar of Ancient Egypt has been used several times for checking the value of the radiocarbon method⁹ although more recently the divergence between the radiocarbon dates and the historical chronology has given grounds for disquiet^{10,11}. The discrepancies are reduced by applying the bristlecone pine calibration to the Egyptian radiocarbon dates¹²⁻¹⁵, but some scholars have suggested that this now makes these Egyptian dates systematically too old instead of too recent. One worker has indeed advocated the calibration of radiocarbon dates from archaeological samples by reference

to the Egyptian dates alone and without regard to the bristlecone pine^{8,16}.

Sufficient data are available to permit a statistical investigation of both problems, taking into account the experimental errors in the radiocarbon determinations. The time range selected covers the period from the beginning of the Egyptian historical chronology (about 3100 BC) to the first secure and astronomically verifiable Egyptian historical date (1872 BC).

Calibration Function

Any calibration curve based on tree rings is derived ultimately from the analysis of a series of tree-ring-dated wood samples, where each sample has both a tree-ring date (generally assumed to establish a date in calendar years with a probable error of less than two or three years), and a radiocarbon date with its attendant standard error arising chiefly from counting problems. Similarly, the historical data used for any check on the dendrochronological calibration arise from a series of samples each having, as well as a radiocarbon date (with standard error), a date in calendar years assigned on the basis of its observed archaeological context and the appropriate historical calendar.

Here we investigate the proposition that the calibration relationship connecting radiocarbon dates and their corresponding "true" dates is the same in both sets of data. If this proposition is correct, it would lend support to the use of bristlecone pine radiocarbon dates to calibrate radiocarbon dates relevant to prehistoric material in general.

Our method assumes that the calibration relationship is of the form $y=f(x)+\epsilon$ where y denotes the radiocarbon date of a sample, x its corresponding "true" date (as determined dendrochronologically or archaeologically), ϵ the random measurement error in the radiocarbon date, and f the unknown "calibration function". This function is approximated by fitting a suitable smoothing function g , using a weighted least-squares procedure. The hypothesis is then tested by standard statistical methods, essentially by comparing the goodness-of-fit of a single smoothing function g fitted to the whole data, with that of two smoothing functions of the same type fitted separately to the two sets of data. If a common curve fits just as well as two separate curves, one may conclude that there is only one calibration curve for both the Egyptian and bristlecone pine data. As the choice of type of smoothing function may be crucial, this method has the important advantage of enabling several possible such functions to be easily compared.

Earlier workers have approached this problem by fitting a smoothing function g to the bristlecone pine data alone, and then matching the observations from individual Egyptian samples against that curve. This can be done in two ways: (a) by comparing, for each Egyptian radiocarbon date, the

corresponding calendar date(s) as calibrated against g with the assigned historical date for that sample; (b) by comparing, for each Egyptian historical date, the corresponding radiocarbon date as predicted by g with the date actually observed (see Fig. 1). In both cases it must be remembered that the smoothing function g is only an estimate of the calibration function f , and therefore has its own associated standard error.

In such a procedure method (b) is preferable to (a) for two reasons. (i) It avoids the possibility of several calendar dates corresponding to a single radiocarbon date if there are "kinks" in the calibration curve (see Fig. 1). (ii) The comparison of predicted and observed radiocarbon dates is preferable to the comparison of predicted and observed calendar dates, for it is the radiocarbon dates which are subject to random errors of known magnitude. The errors associated with the tree-ring dates themselves are thought to be small⁴, whereas those involved in assigning precise historical contexts of samples of Egyptian material are difficult to quantify or treat statistically.

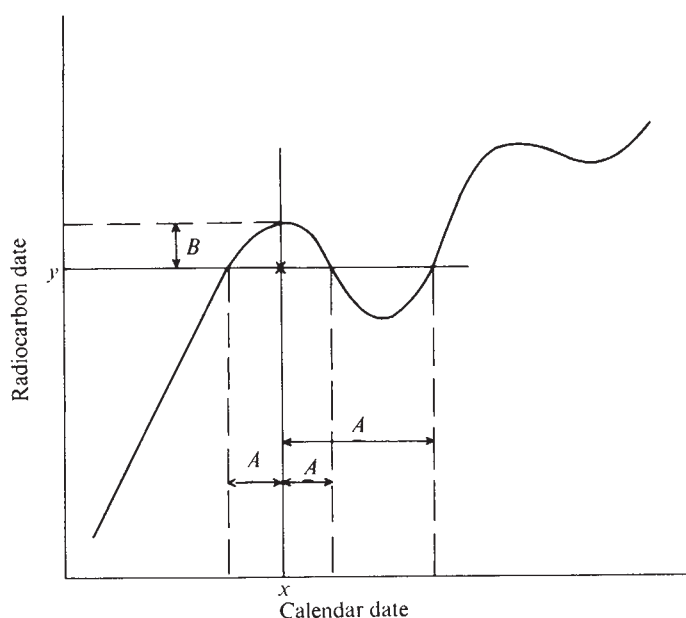


Fig. 1 Methods (a) and (b) of matching a typical Egyptian sample against a fitted calibration curve. Method (a) considers the discrepancies A between the estimated and known calendar dates; method (b) considers the discrepancy B between the observed and predicted radiocarbon date. $\times$, Plotted point corresponding to Egyptian sample; —, calibration curve.

Recent workers^{12,14} using method (a) have not correctly allowed for the standard error of a calibrated radiocarbon date. Such a standard error depends not only on that of the radiocarbon date itself, but also on the nature and precision of the smoothing function near the point of calibration. An important disadvantage of Suess's calibration curve⁵ is that, as it is not determined by explicit reproducible methods, its precision cannot be reliably assessed.

A crucial problem is the choice of type of smoothing function g . Among those already applied to radiocarbon determinations for samples of known age are linear functions¹⁷, periodic functions¹⁸, polynomials¹⁹, and the visual fitting of a subjectively-drawn curve⁵. There is no universally valid criterion for preferring a particular approach, and indeed it is relevant to consider some of the general criteria which might be thought desirable in a smoothing function.

(1) It should be derived by a reproducible and non-arbitrary method.

(2) There should be an objective method of testing whether the curve is an adequate fit to the data.

(3) The fitted smoothing function should in fact be an adequate fit to the data.

(4) There should be an objective estimate of the error in the fitted smoothing function.

(5) The smoothing function need not, however, possess the same general properties (such as continuity, smoothness) as the calibration function, although they may be desirable.

The choice of smoothing function will naturally depend on the purpose of the smoothing. In the case presented here, the smoothing is intended to facilitate the comparison of the Egyptian and bristlecone pine dates. We are not claiming to have produced a universal all-purpose calibration curve relevant to this time period. The production of a suitable calibration curve for use by archaeologists is a related problem at present under investigation. The physical background may well require that the calibration function f should have a bounded continuous first derivative, while the smoothing function g need not necessarily be constrained to have this property. For the present purpose, a discontinuous function, such as a series of unconnected straight lines, may be statistically convenient and quite valid, even if it would not be satisfactory as a calibration curve.

Evaluation of the Data

The data for our analysis consisted of the assigned historical dates, and the corresponding radiocarbon dates with their associated standard errors, for fifty-two Egyptian samples relevant to the time period 3100–1800 BC (calendar years), comprising fifty-one radiocarbon dates given by Berger²⁰ and Edwards²¹, with the subsequent corrections²² noted, and one extra sample (BM 236) found to be paired with UCLA 1208 (ref. 22). We also used the tree-ring date and radiocarbon date corresponding to each of fifty-seven specimens of bristlecone pine relevant to this time period, obtained from the list set out by Houtermans¹⁸. These specimens were dendrochronologically dated by the Laboratory of Tree-Ring Research, University of Arizona, and their radiocarbon dates determined by Suess at the La Jolla laboratory. Concerning the standard errors of these radiocarbon measurements, Houtermans¹⁸ states only that they range from 4 to 7 per mil, and so we decided, in the absence of more specific information, to assign to these radiocarbon dates a common standard error of 5.5 per mil or 45.5 yr, the midpoint of the range.

In constructing a calibration curve, or comparing one proposed curve against another, the magnitude of the errors of measurement is of course important, as this determines how well a given smoothing function g approximates the unknown calibration function f . So, before fitting any smoothing function, it is essential to verify the quoted measurement errors whenever possible.

The standard error of 45.5 yr assigned to the La Jolla radiocarbon dates is at least partially verified by the estimated error variance, namely 2,263 on eleven degrees of freedom, obtained by Clark and Renfrew¹⁷ by considering the differences²³ between replicate radiocarbon determinations carried out by the La Jolla laboratory on samples of wood from Auvernier, Switzerland. These Auvernier samples, however, were not of wood of the bristlecone pine, and so the quoted variance may not necessarily represent the precision of the La Jolla laboratory with bristlecone pine samples.

As many of the Egyptian samples were divided and analysed by both the BM and UCLA laboratories, the comparison allows the evaluation of interlaboratory variations, and hence of the published standard errors which the laboratories associate with their determinations.

Examination of the twenty-five paired differences so obtained showed that there was no systematic difference between these laboratories, but that the paired differences showed significantly greater variation than that expected due to measurement errors, the corresponding χ^2 value being significant at the 0.2% level. This implies that either the real measurement errors are greater than believed, or that there were some gross (non-counting) errors in some of the dates, or both. The standard errors of measurement would have to be 20% to 40% greater than the quoted standard errors in order to explain the full observed discrepancy.

McKerrell⁸ has also concluded that there is no evidence of a systematic difference between these laboratories, but failed to note the excessive variation in the paired differences. The inclusion of two pairs omitted by McKerrell and the use of a more sensitive test does, however, document the excessive variability in the paired differences.

Some impression may be gained of the reliability of the ascription to the samples of a historical context by the Egyptologists concerned, together with the variation in the age of the samples when buried, by comparing the measured radiocarbon dates of samples from different archaeological contexts reported as approximately contemporary. Several analyses are available from different contexts assigned archaeologically to the same period. If these archaeological assignments are correct, the differences in the radiocarbon dates for them should be no greater than would arise from the laboratory measurement errors alone. The question here is not simply the major one of the validity of the Egyptian historical calendar^{11,21,24} but the more practical one of the precision with which these historical dates may be assigned, assuming for the moment their correctness, to the specific samples in question, on the basis of their stratigraphic contexts.

Ten groups of dates were considered, where all the samples within each group were supposedly of the same historical date, and their radiocarbon dates determined by the same laboratory. In three of these groups, the range of the corresponding radiocarbon dates was significantly greater than could be explained by measurement errors. Table 1 summarizes the results of the ten significance tests performed using Table 22 of Pearson and Hartley²⁵.

Table 1 Significance of Range of Radiocarbon Dates of Supposedly Contemporaneous Egyptian Samples

Historical date	BM	UCLA
3025 BC	* (4)	—
3000 BC	—	ns (2)
2950 BC	ns	—
2650 BC	† (4)	ns (2)
2600 BC	ns (3)	—
2335 BC	ns (2)	ns (2)
2000 BC	ns (5)	‡ (4)

—, No dates available; ns, not significant at 5% level; *, significant at 5% level; †, significant at 0.1% level; ‡, significant well beyond the 0.1% level (about 0.001%). Number in parentheses is number of samples in each group.

These results imply (i) that some of the measurement errors are greater than indicated by the laboratories, or (ii) that certain groups of samples are not in fact contemporaneous (which was assumed in these tests), or (iii) there are gross errors in some of the radiocarbon dates. If the measurement errors were increased by 40%, as suggested previously, the two most significant groups would still show significantly large ranges. The observed discrepancies cannot be explained simply by

increasing the standard error of measurement to the extent suggested by the interlaboratory comparison. But the four samples given an historical date of 2650 BC and the five corresponding to 2000 BC were of different materials (such as wood, linen, reed), and these samples might not be of identical age, even when they were buried.

It is concluded that some of the information concerning the fifty-two Egyptian samples is grossly in error: either some "true" dates, or some radiocarbon dates, or their associated standard errors. This has not previously been demonstrated by a systematic statistical analysis, although Michael and Ralph²⁶ and Säve-Söderbergh and Olsson²⁷ have used qualitative comparisons to draw similar conclusions, the latter pointing to uncertainties in some cases about the archaeological date. There does not seem to be any independent objective method of deciding, on the basis of existing data, which pieces of information are wrong and by how much. We suspect that in some cases difficulties in stratigraphic interpretation have led to error. Admittedly, the assigned Egyptian historical dates are not claimed to be exact, but there seems to be no objective way of quantifying their lack of precision.

These results alone show clearly that the attempt to use the Egyptian historical dates as the basis for a "calibration" of the radiocarbon chronology, quite independent of the bristlecone pine dendrochronology¹⁶, is not well founded.

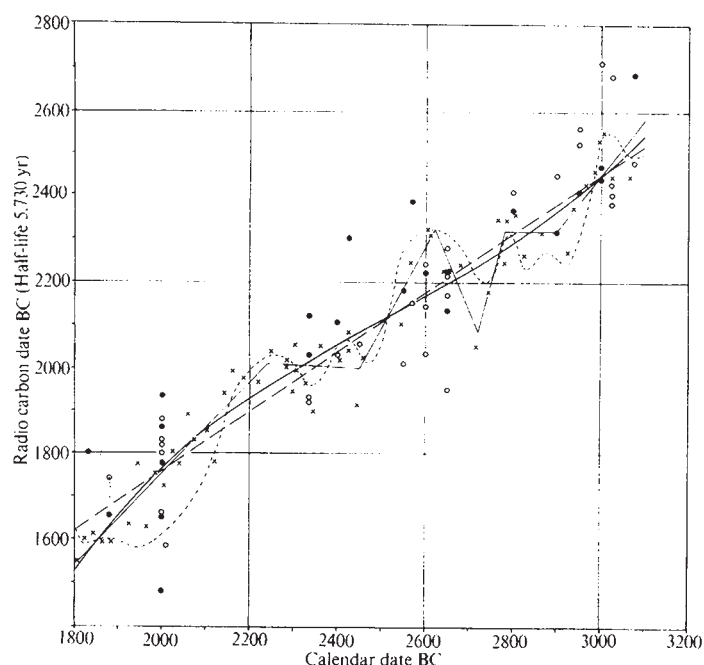
These discrepancies make the Egyptian data less suitable for a check on the bristlecone pine calibration than might be hoped. Both Berger²⁰ and McKerrell¹⁶, however, have based comments on the calibration upon an examination of these Egyptian data, and it is important to consider such claims. In what follows analyses are therefore conducted on the provisional assumption that the assigned historical dates for the Egyptian samples are without error, and that the discrepancies observed are simply the result of high measurement errors of their radiocarbon dates. Although, as already noted, this assumption is not strictly warranted, it should not affect the conclusions unless the historical dates are very seriously in error, as the F-values in Table 2 are not near the critical values.

Statistical Analysis

The problem of testing the hypothesis of a common calibration curve for the bristlecone pine and Egyptian data can be recognized as a special case of testing a sub-hypothesis in a general linear model, for which standard statistical techniques are available²⁸. In order to apply these techniques, it was necessary to assume, at least provisionally, that the calendar dates assigned to all Egyptian and bristlecone pine samples were without error, and that the errors of measurement of the radiocarbon dates were normally distributed. It was also assumed that the standard errors of all radiocarbon dates used were equal to k times the corresponding reported standard errors. The factor k , initially unspecified but later estimated from the data, allows for possible underestimation by all three laboratories of their overall measurement error. As has been noted elsewhere^{29,30}, the reported standard error refers only to "counting error", which is only part of the overall measurement error. The preceding analysis of paired differences indicates that such underestimation of the real measurement errors could be substantial.

Two types of smoothing function g were fitted to the data over the range 3100–1800 BC, using the method of weighted least-squares to allow for the differing precision of the radiocarbon measurements. As the weights used in this procedure depend only on the relative magnitudes of the standard errors of the radiocarbon dates, the weights can be chosen without knowing the actual value of the factor k . First, a single m th degree polynomial was chosen, giving in fact fourteen curves corresponding to the various values of m up to a maximum of

Fig. 2 Relationship between radiocarbon dates and calendar dates, showing the curve published by Suess ---, and three of the smoothing functions fitted by weighted least-squares: —, straight line; —, fourth-degree polynomial; —, piecewise linear curve. Plotted points represent data used in this analysis: ×, bristlecone pine (LJ); ○, Egyptian (BM); ●, Egyptian (UCLA).



14 imposed by the limits of computer accuracy. Second, g was assumed to comprise seven straight lines constrained to be continuous at the six corresponding join-points (see Fig. 2). The number of line-segments was restricted to seven because of computer capacity and the location of the resulting six join-points was estimated statistically without reference to Suess's curve.

Amongst the Egyptian data, in most cases there was more than one radiocarbon date corresponding to each of the eighteen distinct historical dates. For the purposes of curve-fitting, each group of radiocarbon dates corresponding to each historical date could be replaced by a weighted mean radiocarbon date. The residual variation after fitting any smoothing function g to these data can therefore be partitioned into two components. The first component involves the deviations of the weighted groups-means from the fitted function, whereas the second component represents the within-groups variation for these radiocarbon determinations of supposedly contemporary Egyptian samples. Being the same for all fitted functions, this second component measures the intrinsic variability of the Egyptian data, which no amount of curve-fitting can reduce.

The corresponding analysis of variance is given in Table 3 for the case where the function g is simply a straight line. The

weighted least-squares procedure is exactly equivalent to transforming the data so that the transformed radiocarbon dates have a common error variance and then performing a standard least-squares analysis of the transformed data. In this particular case, the weights were chosen so that this common error variance was equal to $(45.5 k)^2$. Item e in Table 3 is an unbiased estimate of this variance, even if the fitted straight line is not an adequate fit to the data and even if our hypothesis is incorrect. It follows then that the factor k must be at least 1.5 and possibly 1.8, implying that the true standard errors of the radiocarbon dates must be at least 50% and possibly 80% greater than the quoted standard errors. This reinforces the doubts expressed earlier concerning the validity of the Egyptian data.

In Table 3, comparison of items d and f with e , using the appropriate F -tests, shows that a straight line is in fact an adequate fit over the time range to both the Egyptian and bristlecone pine data separately. Moreover, comparison of items g and e , yielding the low F -value of 1.08, indicates that a single straight line fits the combined data just as well as these two separate lines. In other words, the hypothesis of a common calibration curve (in this case, a straight line) is well supported.

Table 2 Comparison of Goodness-of-Fit of Various Smoothing Functions Applied to Egyptian, Bristlecone Pine and the Combined Data

Type of curve	Bristlecone pine data		Egyptian data		Combined data		F -value	Degrees of freedom
	Residual s.s.	m.s.e.	Residual s.s.	m.s.e.	Residual s.s.	m.s.e.		
Linear	310,319.72	75.11	337,186.78	82.12	661,938.08	78.65	1.08	2,34
Quadratic	291,545.86	73.47	333,640.54	82.51	657,691.58	78.76	1.63	3,34
Cubic	266,432.82	70.90	333,599.58	83.37	627,952.14	77.33	1.05	4,34
Quartic	266,371.64	71.57	320,793.39	82.62	625,044.44	77.52	1.14	5,34
Eighth degree polynomial	214,232.53	66.80	298,367.62	83.30	602,018.40	77.58	1.49	9,34
Fourteenth degree polynomial	167,840.19	63.21	270,739.99	85.54	543,762.94	76.05	1.05	15,34
Seven connected lines	151,717.62	55.64	300,483.77	82.64	539,256.58	73.07	1.64	8,34

Residual s.s., weighted residual sum of squares; m.s.e., mean-square-error. F -value is the value of the standard F -statistic for testing the hypothesis of a common calibration curve of the given type. Items a , b and c of Table 3 are given in the first, third and fifth columns respectively.

Similar tables of analysis of variance may be constructed for each fitted smoothing function, simply by substituting the value of items a , b and c given in Table 2, and using the well-known structure of analysis-of-variance tables. The resulting F -values for testing the hypothesis of a common calibration function are listed in Table 2. In all the cases considered, this hypothesis was supported, as testified by the low F -values. Table 2 also gives a comparison of the goodness-of-fit of the various smoothing functions when fitted to the combined data or to either the bristlecone pine or Egyptian data separately.

If the assigned historical dates of the Egyptian samples were not correct, then the various F -statistics would have only an approximate F -distribution, rather than the exact F -distribu-

comparison of the two time scales. We claim, however, that our analysis is the most precise comparison possible in the circumstances. A more exacting test of the comparability of the two data sets could only be obtained with better data, and in particular better Egyptian data.

Nonetheless, although the comparison is not as precise as is desirable, the available information indicates that the hypothesis of a common calibration curve for the bristlecone pine and Egyptian data over the time period 3100 to 1800 BC is not contradicted, and suggestions to the contrary are not well founded. The conjunction of the bristlecone-pine-calibrated Egyptian radiocarbon dates and the historical dates for Ancient Egypt from 3100 to 1800 BC carries with it the implication that, within the error limits discussed, both chronological systems are correct. It seems unlikely that the Egyptian historical calendar, as reconstructed by Egyptologists today, and the bristlecone pine calibration of radiocarbon dates should be in error in precisely the same way over this range, so as to yield closely comparable yet erroneous calibration functions.

Our analysis thus lends support to the general validity of both the radiocarbon calibration and the Egyptian historical chronology. Further accurate work on Egyptian material is still required to reduce the level of error and thus to make the comparison more sensitive. But the present harmony gives some grounds for optimism concerning the validity of the bristlecone pine calibration as applied to prehistoric studies in general.

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Table 3 Analysis of Variance due to Fitting Linear Smoothing Functions

Source of variation	Degrees of freedom	Sum of squares	Mean-square
Deviation of weighted group means from Egyptian line	16	111,724.76	6,983 d
Within-groups (Egyptian)	34	225,462.02	6,631 e
Total residuals from Egyptian line	50	337,186.78 b	6,744
Residuals from bristlecone pine line	55	310,319.72 a	5,642 f
Difference between lines	2	14,431.58	7,216 g
Residuals from common line	107	661,938.08 c	
Regression due to common line	1	5,895,145.74	
Total	108	6,557,083.82	

tion assumed in the preceding significance tests. But, provided these dates are not very seriously in error, our conclusions remain the same, because the computed F -values are comparatively distant from the critical values. As there is no objective measure of the precision of these estimated dates, no completely objective statistical test of the hypothesis is possible, using the given data. Method (a) seems to overcome the problem by calibrating the Egyptian radiocarbon dates using a calibration curve based on bristlecone pine observations only, but there is then no objective way of comparing those calibrated dates with the assigned historical dates. Some assumption concerning the precision of these dates is thus necessary for any analysis, and our assumption facilitates the comparison of several smoothing functions. The remaining assumptions are likely to be at least approximately correct and, in any case, the analysis would be only marginally affected by small departures from them.

Validity of the Time Scales

Detailed analysis of the Egyptian data shows that, if major experimental errors be discounted, either the standard errors associated with the radiocarbon dates have been grossly underestimated, or some of the calendar dates assigned to samples on stratigraphic evidence are seriously in error, or both. It would therefore be ill-advised to use the Egyptian data to construct a calibration curve independent of the bristlecone pine chronology, although this has been suggested by McKerrell.

These deficiencies in the Egyptian data unfortunately preclude any completely objective statistical analysis, or any exact

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Turning the Left Cheek

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This article explores the fact that portrait painters have tended to paint the left cheek rather than the right one.

THE "likeness" of a person's face is seldom well conveyed when the profile is not shown, and artists have usually portrayed their subjects facing to one side. Which side? Why should it matter: the right cheek is generally as representative of the man as is the left? Yet analysis of actual portraits shows there to have been a consistent tendency to paint the left cheek rather than the right. Moreover the tendency is significantly more marked both in portraits of women than of men and in portraits showing the subject's body than in those showing just his face.

The material for this study was 1,474 painted portraits, produced in Western Europe from the sixteenth to the twentieth centuries. The sources of these portraits were: the National Portrait Gallery, London (571 portraits), a textbook on Elizabethan and Jacobean portraiture¹ (338 portraits), the portraits and portrait miniatures of the Fitzwilliam Museum, Cambridge (231 portraits), a miscellaneous collection of art books belonging to the first author (291 portraits) and the exhibition at the National Portrait Gallery entitled "The Masque of Beauty" (August 1972) (43 portraits).

All portraits showed a single person only. For each the following points were noted: the sex of the subject; the side of the subject's face which was turned towards the viewer; and the amount of the subject's body which was visible in addition to his head. Rare cases of full-face portraits were excluded from the study.

Table 1 Data by Sex

	Left	Right	% left	P
Men	524	408	56	<0.001
Women	376	175	68	<0.001

Of the 1,474 portraits 891 showed more of the left cheek than the right. This 60% bias to the left is highly significant ($P < 0.0001$ on a χ^2 test). Table 1 shows the data broken down by the sex of the subject. Although the left-cheek bias is significant in portraits of both men and women, the bias in women's portraits (68% to the left) is much greater than in men's (56% to the left), the difference being significant at the 0.001 level.

Analysis of the data in terms of the amount of body visible is shown in Table 2. Here the portraits have been subdivided into two groups, "head only" or "head and body", according to whether or not any of the body below the shoulders was portrayed. For both men and women the left-cheek bias is much stronger when the body below the shoulders is included in the picture. Taking both sexes together, the difference between the "head only" group (51% to the left) and the

"head and body" group (65% to the left) is significant at the 0.001 level.

Several explanations may be offered for these findings. For instance, there might be bias in the artist's skill. Most artists are right-handed and it might be easier for a right-hander to draw a profile to the left of the canvas (as it is easier to write from left to right).

Second, there could be bias in the positioning of the subject relative to the artist. A right-handed artist commonly holds the palette in his left hand so that his face, the palette and the subject's face are all in line and matching of colours from the palette is facilitated. The easel is usually to the right of him. Thus the artist is constrained to take up a position such that his left cheek faces the subject, and this position might be reciprocated by the subject to strike a balance.

Third, there might be bias in the social interaction between the subject and the artist. Independently of the constraints considered in the previous paragraph there might be a general (unexplained) tendency for any two people to feel more comfortable when they are positioned in such a way that their left cheeks face each other.

Table 2 Data in Terms of Body Visible

	Men				Women			
	Left	Right	% left	P	Left	Right	% left	P
Head only	142	166	46	NS	101	66	60	<0.01
Head and body	382	242	62	<0.001	265	111	70	<0.001

Fourth, there might be some visual preference for the left side of the face; perhaps it is considered more attractive than the right. Such a preference could possibly arise from some form of "exposure learning" whereby people come to prefer familiar visual stimuli²: Salk³ claims that mothers tend to carry their babies on their left breast, so that a baby is exposed predominantly to the mother's left cheek. Alternatively the preference might reflect genuine differences between the left and right sides of the face resulting, perhaps, from fashion in make-up or coiffure (for example, parting of the hair over the left temple or placing beauty spots on the left cheek?).

Fifth, there may be superiority of the left visual half-field in facial recognition. Clinical evidence suggests that the right cerebral hemisphere (hence the left visual half-field) is superior to the right in recognizing pictures, especially pictures of faces⁴. A profile drawn to the left of the canvas might thus in a sense be more readily perceived.

Sixth, the way in which people turn their heads may be biased in favour of one direction. There might be a simple motor bias such that when the head is turned aside it is more likely to be turned to the subject's right than to his left. Informal observations do suggest that when people shake their heads (as if to say no) they tend to make the first move to the right.

But the data do not give much support to any of these explanations. The difference between men's and women's portraits cannot be accounted for by any of the hypotheses except maybe the fourth. The difference between "head only" and "head and body" portraits is contrary to the predictions

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of the first hypothesis and cannot convincingly be accounted for by any of the others.

Perhaps the explanation in fact lies within the conventions of painting itself. Artists communicate more than mere physical likeness with their pictures: they attempt to convey character and status, and to do so they resort in part to arbitrary "signs". Baden-Powell instructed Boy Scouts to shake hands with their left hands in order to set themselves apart from other people: maybe when an artist paints his subject facing either to the left or to the right he is in some similar way imposing a structural classification which, as it were, puts the subject in his place. That such a system of signs should be unacknowledged either by the sender or receiver of the "message" would have parallels in other systems of human communication⁵.

This last possibility, in some ways the most promising, is unfortunately the hardest to test experimentally. A semeiological analysis of the kind which has been successfully applied to symbolism in other forms of art⁶ is unlikely to be fruitful when, as with the portraits of this study, too little is known *post hoc* of what message the artist intended to put over. An indirect test of the existence of a left/right sign system (which might also be used to test for left/right "preferences") would be to make mirror-image reproductions or unfamiliar portraits and to compare people's reactions to the original and its mirror-image with a technique such as the "semantic differential"⁷. A difference in people's attitudes to the same portrait reversed from left to right would argue strongly that the significant bias found in actual portraits is significant precisely because it does in fact signify.

Certainly one does not have to look far for anecdotal evidence that people have strong feelings about which side of their own face they would wish to put on show. The Royal Mint has recently revealed that when coin designs were first produced for King Edward VIII (late Duke of Windsor), he objected to those which showed the right side of his face. His misgivings, says the Royal Mint's librarian and curator, G. P. Dyer, arose from a firm conviction that the features on the left side of his face were superior to those of the right⁸. Superior is an interesting choice of word. It would appear from the original designs that the two sides of the King's face were, except for a left-side parting, almost perfect mirror images. Who knows but that it is a mark of a superior person to turn the left cheek to the world?

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James I



Elizabeth I

Rifamycin Biosynthesis Studied with ^{13}C Enriched Precursors and Carbon Magnetic Resonance

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Studies using ^{13}C -enriched precursors in combination with carbon magnetic resonance spectroscopy have established the biosynthetic origin of most of the carbon atoms present in rifamycin S and suggest a common scheme for ansamycin biogenesis.

ALTHOUGH radiotracers, in particular ^{14}C , have been exceptionally useful in the analysis of biosynthetic pathways, they have one severe disadvantage: the necessity of carrying out extensive chemical degradations to establish the location of incorporated isotope. In the case of complex molecules one usually has to be satisfied with a partial analysis of isotope distribution.

The advent of ^{13}C as an alternative to ^{14}C in biosynthetic studies has led to the introduction of a technique which can completely establish the location of isotope within labelled metabolites. ^{13}C is a stable isotope present at 1.1% in natural organic molecules. Several ^{13}C -enriched compounds, suitable for use in biosynthetic studies, are commercially available.

Metabolites labelled with ^{13}C , obtained by adding enriched precursors to cultures, can be analysed by three techniques: mass spectrometry¹; proton magnetic resonance spectroscopy²⁻⁷ (PMR); and carbon magnetic resonance spectroscopy⁸⁻¹⁷ (CMR). The most important of these approaches is the last one¹⁸. It is made possible by the fact that the nucleus of ^{13}C , unlike that of ^{14}C , has magnetic properties; also, recent advances in NMR instrumentation, in particular the introduction of the pulse and Fourier transform method, enable good natural abundance CMR spectra of most organic compounds to be obtained. Such spectra can be simplified by complete decoupling of ^{13}C from ^1H , all carbon atoms then appearing as single lines. The site of incorporation in a ^{13}C -labelled metabolite is revealed by an increase in peak height on its CMR spectrum compared with that obtained on the natural abundance spectrum.

Rifamycin Biogenesis

The rifamycins constitute a family of antibiotics first isolated in the Lepetit Research Laboratories in 1957 (ref. 19). Chemical²⁰ and X-ray crystallographic²¹ studies showed that they consist of a naphthoquinone chromophore spanned by an aliphatic bridge, called the ansa chain (Fig. 1); they were

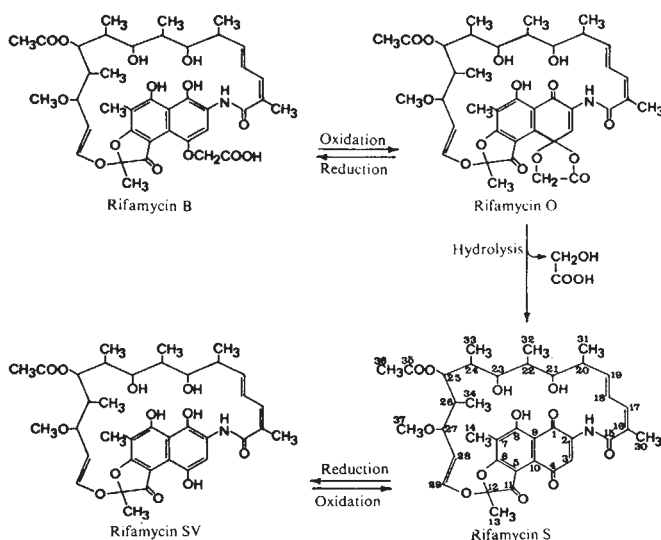


Fig. 1 Structural interrelationships of rifamycins B, O, S and SV.

the first examples of a new class of secondary metabolites for which Prelog has proposed the name ansamycins. Preliminary data (unpublished) of ^{14}C -precursor incorporation obtained in Prelog's and our laboratories led Corcoran²² to propose a scheme for rifamycin biosynthesis in which the ansa chain was composed of five *propionate* and two *acetate* units. (Whenever a substrate is written in italics it indicates the chemical

entity which actually participates in the biological reaction.) The origin of the chromophore was not clear but appeared to contain only two *propionates*; the remainder was assumed to derive from *acetate*. Analysis of ^{14}C and ^3H -propionate incorporation has now been completed by Prelog (unpublished results) and only partially confirms Corcoran's proposal.

Incorporation of ^{13}C -Acetate and ^{13}C -Propionate

In the experiments described here, samples of rifamycin B enriched with ^{13}C were prepared by addition of ^{13}C -propionate and ^{13}C -acetate to fermentations of *Nocardia mediterranei*,

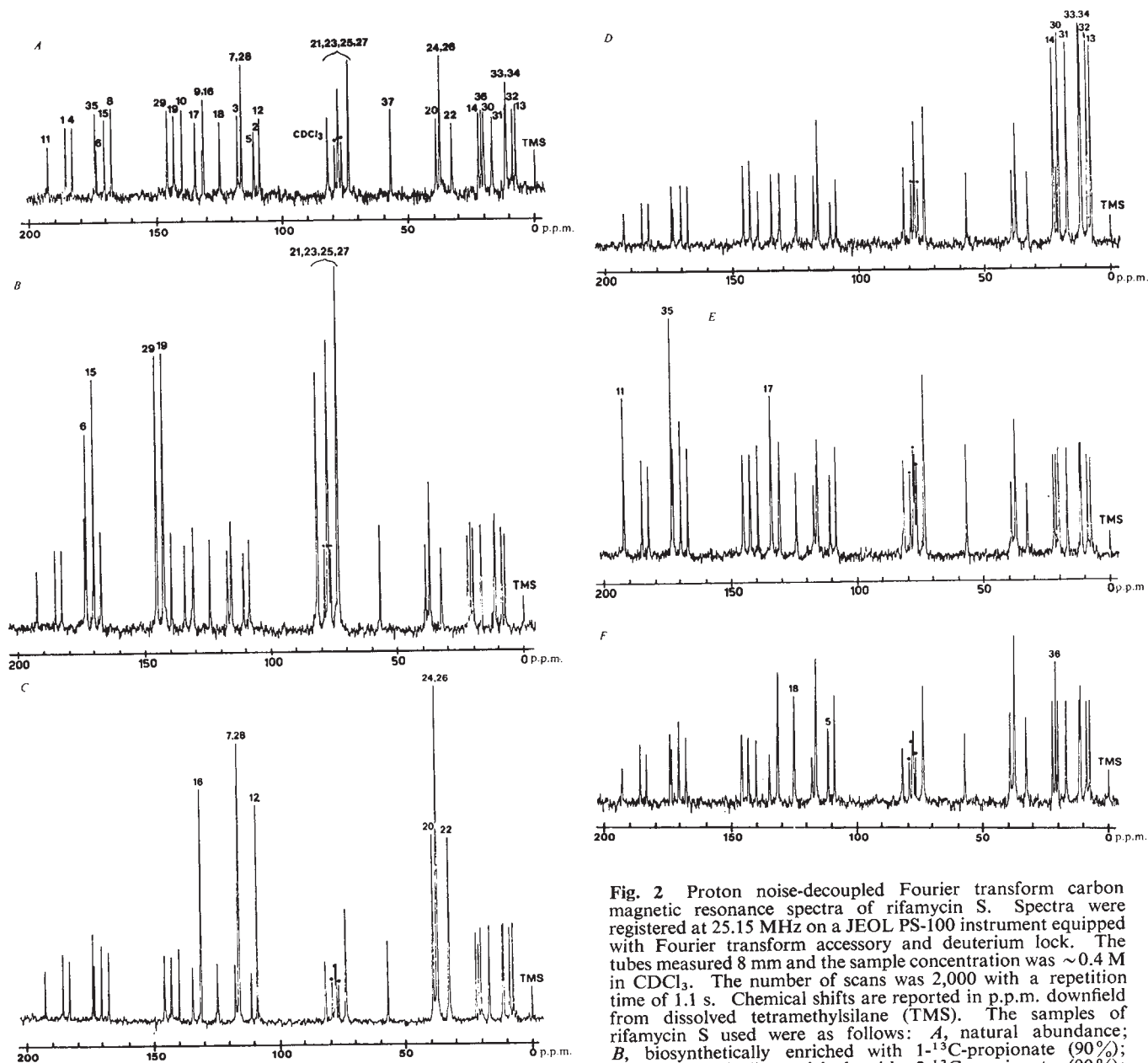


Fig. 2 Proton noise-decoupled Fourier transform carbon magnetic resonance spectra of rifamycin S. Spectra were registered at 25.15 MHz on a JEOL PS-100 instrument equipped with Fourier transform accessory and deuterium lock. The tubes measured 8 mm and the sample concentration was ~ 0.4 M in CDCl_3 . The number of scans was 2,000 with a repetition time of 1.1 s. Chemical shifts are reported in p.p.m. downfield from dissolved tetramethylsilane (TMS). The samples of rifamycin S used were as follows: A, natural abundance; B, biosynthetically enriched with 1- ^{13}C -propionate (90%); C, biosynthetically enriched with 2- ^{13}C -propionate (90%); D, biosynthetically enriched with 3- ^{13}C -propionate (64%); E, biosynthetically enriched with 1- ^{13}C -acetate (90%); F, biosynthetically enriched with 2- ^{13}C -acetate (90%). Precursors enriched with ^{13}C were all purchased from Merck Sharp and Dohme Ltd, Montreal, Canada.

Although rifamycin B is the normal fermentation product, biosynthetic studies²³ have centred on the simpler rifamycin S, which is the precursor of this family of antibiotics^{24,25} and which had previously been obtained from rifamycin B by successive oxidation and hydrolysis, with the intermediate formation of rifamycin O (Fig. 1). Simple reduction of rifamycin S yields rifamycin SV.

carried out according to known procedures²⁶. Concentrations of more than $200\ \mu\text{g ml}^{-1}$ of acetate and propionate are toxic, and it proved necessary to pulse-feed precursors twice a day over 96 h to obtain a satisfactory incorporation. At the end of the fermentation ^{13}C -enriched rifamycin B was isolated, converted to rifamycin S and then examined by CMR spectroscopy.

The natural abundance proton-decoupled CMR spectrum of rifamycin S is shown in Fig. 2A; the attributions given will be the subject of a separate publication³⁴.

Assignments within the two groups of carbon atoms C-20, C-22, C-24, C-26 and C-31, C-32, C-33, C-34 are only tentative and those within a third group have not been established. Conclusive assignments within these three groups must await the appropriate selective proton decoupling experiments, but fortunately none of these uncertainties affects the biosynthetic argument as the members within each group have the same metabolic origin.

The CMR spectra of the five samples of labelled rifamycin S obtained from the different ¹³C-enriched precursors are shown in Fig. 2B–F. Peak heights on spectra of the propionate-labelled samples show that: carbons 6, 15, 19, 21, 23, 25, 27 and 29 derive from C-1 of propionate; carbons 7, 12, 16, 20, 22, 24, 26 and 28 derive from C-2 of propionate; carbons 13, 14, 30, 31, 32, 33 and 34 derive from C-3 of propionate. Thus, there are eight *propionate* units in rifamycin S, one of which has lost its methyl (from C-28), during biosynthesis.

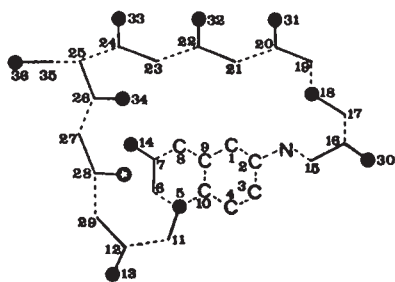


Fig. 3 Alignment of acetate and propionate residues in rifamycin S. Filled circles represent the methyl groups of the propionate (●—) and acetate (●—) residues. Bonds joining the carbon atoms of subunits are shown as solid lines and bonds joining the subunits as broken lines. The "odd" propionate methyl on C-28 (●) is lost during biosynthesis.

Interpretation of the spectra obtained for rifamycin S biosynthetically enriched with ¹³C-acetate is complicated by the concurrent, indirect incorporation of the precursor through *propionate*. This is particularly evident in the case of 2-¹³C-acetate where many carbons are enriched. Comparison with the ¹³C-propionate spectra reveals that all carbons enriched by 2-¹³C and 3-¹³C-propionate are also enriched by 2-¹³C-acetate. This is in agreement with the expected metabolic conversion of acetate to *propionate* during the Krebs cycle. In addition to this indirect incorporation three carbons (C-35, C-17 and C-11) are enriched by 1-¹³C-acetate and three (C-36, C-18 and C-5) by 2-¹³C-acetate.

Although quantification of data obtained on proton-decoupled spectra is made difficult by the nuclear Overhauser effect²⁷, an approximation of the degree of enrichment in a particular carbon atom can be estimated from peak heights on enriched and natural abundance spectra. When this is done for the six acetate-derived carbons, those of the acetoxyl group (C-36, C-35) appear more enriched than the other two units (C-18, C-17 in the ansa chain, and C-5, C-11 in the chromophore). This is shown in Table 1, where the data for a representative *propionate* unit are included for comparison. These data are in agreement with a different metabolic origin for the acetoxyl and the other two units. Secondary metabolites containing *acetate* and *propionate* units are probably synthesized in the same way as fatty acids; that is acetyl CoA and propionyl CoA function as initiators whereas malonyl CoA and methyl malonyl CoA participate in chain extension²⁸. The two *acetate* units C-5, C-11 and C-18, C-17 are clearly in chain extension situations and should derive from malonyl CoA, giving a lower incorporation at these two positions in comparison with the

acetoxyl group which must derive more directly from acetate (by way of acetyl CoA?). Furthermore, incorporation of ¹³C-acetate through *propionate* is of the same order of magnitude as that through malonyl CoA.

Table 1 Estimated Incorporation of ¹³C-Acetate

Carbon atoms	Enrichment factor *	
	2- ¹³ C-Acetate	1- ¹³ C-Acetate
C-5	1.9	1.4
C-11	0.8	2.4
C-18	2.0	1.0
C-17	0.9	2.0
C-36	2.6	1.2
C-35	1.1	3.0
C-30	1.9	1.4
C-16	2.0	1.1
C-15	1.5	1.8

* Peak heights $\frac{\text{enriched sample}}{\text{natural abundance}}$ from spectra run under essentially identical instrumental conditions and corrected for difference in concentration.

No such differences in enrichment are evident if similar calculations are performed for the samples biosynthetically enriched with ¹³C-propionate. When the origin of the twenty-nine carbons deriving from propionate and acetate is summarized (Fig. 3), it can be seen that none of the *propionate* units is in a chain initiating situation. Therefore we assume that all propionate incorporation occurs through methyl malonyl CoA; in fact ¹⁴C-methyl-malonate gives essentially the same ratio of labelling for ansa and chromophore as ¹⁴C-propionate (unpublished results).

Direction of Chain Growth

Figure 3 shows that the ansa chain can be made by a regular, clockwise condensation of *propionate* and *acetate* units; this is specified by the orientation of the *acetate* unit whose methyl group gives rise to C-18. In this respect the attribution of C-17 and C-19 was crucial, as an interchange would indicate the reverse direction of chain growth during biosynthesis. It was therefore unambiguously established on the basis of specific proton decoupling experiments.

The ansa chain is interrupted between C-12 and C-29 by an ether linkage. The unusual labelling pattern and the fact that the *propionate* and *acetate* units in the ansa chain and chromophore are equally labelled suggest that C-13, C-12 and C-29 originate from a single propionate unit subsequently split by the introduction of an oxygen atom. In this way it is possible to construct rifamycin S from a single polyketide chain, initiated by a seven carbon aromatic moiety.

Origin of Remaining Carbon Atoms

Earlier work with ¹⁴C showed that the methoxyl group C-37 comes from methionine along the normal, folate-mediated pathway (unpublished results). Thus we are left with seven carbons and a nitrogen of unknown origin. Our results disagree with the recent suggestions²⁹ that the naphthoquinone moiety arises by condensation of mevalonate with a phenolic compound and that it is synthesized separately from the ansa chain. Surprisingly, the acetate-plus polymalonate pathway, a common means of aromatic biosynthesis in the Actinomycetales, is not responsible for production of rifamycin chromophore as only one *acetate* unit is present. We have

been unable to obtain a specific incorporation of other known ^{14}C -labelled precursors of aromatic rings, such as shikimate, into this part of the molecule.

A Common Ansamycin Progenitor?

Two other families of ansamycins with naphthalenic chromophores are known: tolypomycins³⁰ and streptovaricins³¹. Many of the differences between and within these groups of antibiotics can be recognized as variations commonly seen among metabolites deriving from the same progenitor. But certain differences seemed indicative of a different sequence of building blocks in the original chain; thus C-28 and C-29 in the rifamycins and tolypomycins appeared to derive from *acetate* whereas the analogous carbons in the streptovaricins clearly come from *propionate*. Our results show that all three types of ansamycin can derive from the same fundamental molecule.

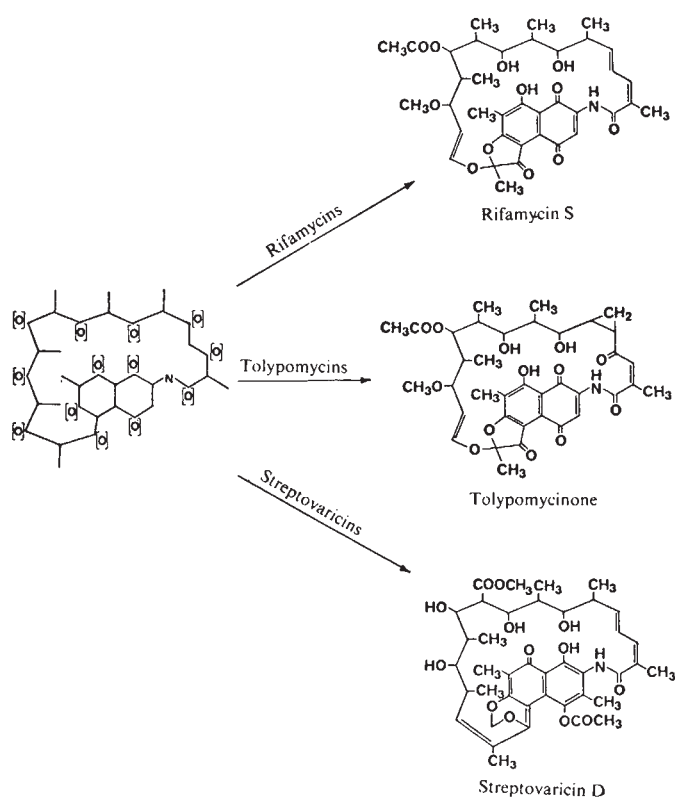


Fig. 4 Carbon skeleton of hypothetical common progenitor and its relationship to the naphthalenic ansamycins. Oxygen functions are bracketed as a result of uncertainties in the timing of their introduction or modification.

The carbon skeleton of the hypothetical progenitor and its relationship to the naphthalenic ansamycins is shown in Fig. 4. While the streptovaricins maintain all the fundamental features of this precursor, the tolypomycins and rifamycins would be formed by introduction of an oxygen between C-12 and C-29, and loss of the methyl from C-28 (the numbers refer to those given for rifamycin S in Fig. 1). Enzymes involved in the synthesis of secondary metabolites quite often lack the absolute specificity characteristic of primary metabolism, making it difficult to establish an unambiguous sequence for the biosynthetic reactions. Thus, some of the subordinate modifications, such as introduction of double bonds or methylation at C-3, may occur before or after completion of the carbon skeleton.

Two ansamycins characterized by benzenic chromophores have recently been discovered, geldanamycin³² and maytansine³³ (see Fig. 5), the latter, in contrast to all other ansamycins, is a plant product. In both cases, it seems that a similar scheme of biogenesis operates and that the precursor of the aromatic part is the same as that for the other ansamycins. It is tempting to suggest that absence of the appropriate oxygen function "ortho" to chain attachment has prevented closure of the second ring and hence formation of a naphthalenic chromophore.

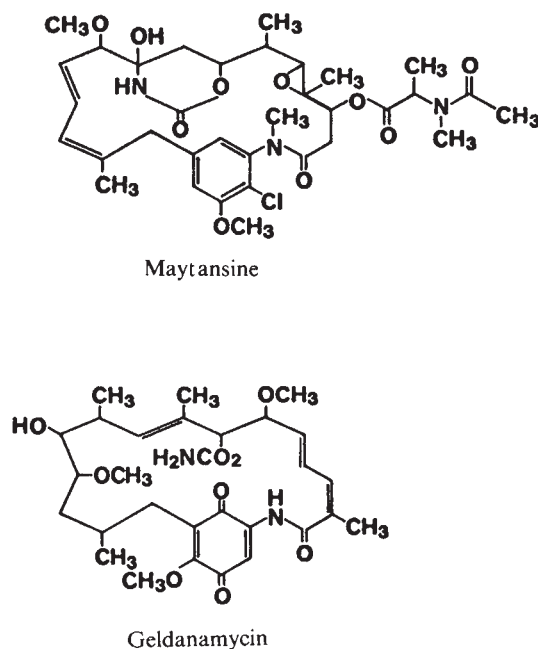


Fig. 5 Structures of the benzenic ansamycins, geldanamycin and maytansine.

Advantages and Limitations of the Technique

The results obtained with rifamycin S illustrate the usefulness of ^{13}C -enriched precursors in conjunction with CMR as a tool for studying the biosynthesis of complex molecules.

Successful application of this approach depended largely on two favourable factors: first, a range of rifamycin derivatives was already available which permitted interpretation of the natural abundance CMR spectrum; second, a high level of precursor incorporation could be obtained. The use of ^{13}C can avoid recourse to the chemical degradations associated with radiotracer techniques, but interpretation of the CMR spectrum may itself require a considerable chemical effort. Although very high levels of incorporation have occasionally been obtained, the extent of enrichment required with ^{13}C will probably limit a generalized application of this approach, and it must be remembered that the metabolic pattern itself may be altered by the substrate amounts of precursor necessary to obtain such an enrichment.

The main outcome of our results is that the ansamycins, which were previously considered to be a group of natural products on purely structural grounds, can now be considered a family from the biosynthetic point of view, deriving from a polyketide chain initiated by the same aromatic moiety. It is doubtful that the same conclusions could have been reached with the conventional radiotracer approach.

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Continental Drift and Reserves of Oil and Natural Gas

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The necessary physiographic location of deltaic sources deposited during the past 200 million years requires that continental movements be taken into account.

ANY new concept which is likely to assist in the location and assessment of the remaining reserves of oil and hydrocarbon gas is of obvious importance so it is opportune to use reviews^{1,2} of the conditions of formation of the principal hydrocarbon sources and their subsequent migration and preservation in reservoirs to see how these are related to movements of the Earth's surface.

Deposition of Organic Substances

The ability of hydrocarbons to migrate³ and their complex chemical nature complicates matters but it is generally agreed that they are organically derived^{4,5}, the oil coming chiefly from animal proteins and the gas including some

vegetal content¹. Sources some 10⁷ yr old are almost entirely deltaic and occur independently of latitude, the organic materials being trapped within the rapidly accumulating sediments. The rich terrestrial vegetation of tropical areas means that there may be some latitudinal control on the percentage of gas to oil, but this is unlikely to be as significant as later factors. The location of deltas today is evident but somewhat older deltas exist which receive little or no drainage today and are therefore harder to distinguish physiographically as continental movements have separated the headwaters from deltas which now lie isolated and unexploited on other continents. The formation of mountain systems, along subduction zones or by continental collisions, also drastically changes the drainage pattern so that, for example, dispersed oil fields of the Andes may once have formed part of the proto-Amazon delta⁶ when the Amazon drained from Africa. Deltaic areas older than 200 m.y., which covers the "Wegenerian" phase of continental drift, are almost impossible to locate physiographically but are of less economic importance because most productive deltaic areas are less than 200 m.y. old and geological identification is possible, irrespective of age.

Shales are generally considered to have the highest initial

organic content, followed by carbonates and finally sandstones—although sandstones are the principal rock type for major reservoirs¹ and can be a significant contributor to their own reservoirs. But the productivity of source rocks depends on the ease of migration of the hydrocarbons so that the most productive source rocks are the massive carbonates, although the productivity of all source rocks is increased by heating. Deep oceanic sediments accumulate slowly and are unable to trap significant protein matter before oxidation so that deep oceans are not potential oil or gas sources, except where shallower sediments have slumped into them, but much of the hydrocarbon is also likely to be lost at this time.

Because more than 90% of known oil and gas fields^{1,7-9} are associated with evaporite deposits the conditions for evaporite accumulation are relevant to the source, migration and preservation by hydrocarbons. Most marine evaporites are formed of anhydrite, with some gypsum, and contain only small amounts of the more soluble halite. Even when halite forms a large part of the deposit, it only represents temporary conditions as it was laid down some 10^2 to 10^3 times faster than anhydrite¹⁰. Significant deposits cannot therefore have formed by prolonged, simple evaporation of seawater because halite would always predominate. Two depositional environments have been postulated to account for these features—the silled basin and the sabkha. The former¹⁰⁻¹² consists of a pool of water into which only surface waters can flow. As these surface waters evaporate, saline brines form and, being denser, sink to the bottom where the least soluble salts are eventually precipitated; but the salinity can only rarely increase to the concentration appropriate to halite deposition. “Sabkha” regions^{13,14} form from the occasional flooding of supratidal flats which create extensive areas of shallow marine waters. As these waters evaporate and retreat, the least soluble salts are left behind. These deposits are greatest where the flats are bordered by lagoons in which the salinity is already higher than normal seawater. Such extensive flat regions would be more common when orogeny was less active than during the past 20 m.y. and when sealevel was more constant than during the eustatic changes of the most recent Ice Age.

In both environments the depositional area must sink relative to the barrier and sealevel to account for the observed thicknesses of evaporites. Rapid subsidence in areas of low evaporation would cause persistent flooding and the solution of previous salts, as would also happen with rare rainstorms in areas of low evaporation and slow subsidence. So although both types of environment could exist temporarily at any latitude, thick deposits could only form in regions with a very high rate of evaporation, that is where hot, dry conditions are accompanied by strong diurnal on-shore and off-shore winds. Cyclotherms and structures in associated sediments indicate intermittent subsidence so that the evaporation rates would have to match the maximum rate of subsidence. Such prolonged subsidence implies movements of the lower crust and upper mantle, as occurs in response to isostatic adjustments, for example during continental separation¹⁵. For continued deposition, the barriers of a silled basin and the shoreline of a sabkha must maintain their position relative to the subsiding depositional area. A fault controlled structure, where very localized tectonic movements happen to cancel out regional movements, could create this situation, but it is more likely that such persistent barriers or shorelines would result from sedimentary or biological processes. Sandbars could be maintained relative to sealevel if marine currents and a sediment supply were unaffected by the subsidence, but the most persistent type of barrier/shoreline would be formed by shallow reef structures. Reefs in low latitudes would easily maintain their position relative to an intermittently deepening sea as their growth is optimized in shallow, warm, clear waters. These low latitude conditions,

necessary for optimum evaporite formation, are bordered by those for the optimum production and preservation of hydrocarbon source materials as the concentration of nutrients in warm, saline waters results in rich organic blooms of microorganisms, the salinity restricts the entrance of larger organisms which could feed on them and the nearby anaerobic bottom conditions are ideal for the preservation of the organic debris¹⁶⁻¹⁸.

Migration

The gradual freeing of hydrocarbons from the original organic matter occurs continuously but is accelerated by small temperature rises^{19,20} to which plate tectonic theories and continental drift have varying degrees of significance. Tectonic heating during collision between two continental blocks is likely to be locally severe and drive off all hydrocarbons, but the less disturbed areas and margins of collision zones may be areas of significant oil and gas migration. Such areas can be located from palaeomagnetic measurements of the angle and rate of collision but the shape of the original edges is also critical and this can only be detected by detailed geological and geophysical studies which, among other things, yield the information of sources, migration and reservoirs. Similarly, in subduction zones there is higher heat flow and igneous activity in a region up to 1,000 km wide, but the distribution of heat is not regular and the local controlling factors are only just becoming clear²¹ and the estimation of heat flow in different areas, now and in the past, should become more precise as such interacting factors are considered further.

The igneous activity and high heat flow along spreading oceanic ridges are not of any importance within the oceans, where there are no source rocks, but become highly significant when these ridges intersect, or have intersected, continental plates. Local destruction of reserves is likely where significant igneous activity was involved, such as at previous plume centres, but these will also be rimmed by high gas pressures. In general, the high heat flow of the ridges will have been a major cause of migration as the hydrocarbons would move slowly from deep to shallow levels as the regional temperature increased. Thus the numerous, highly productive gas fields of north-west Siberia occur over the continental extension of the Arctic ridge; similarly the productive oil fields around Los Angeles²² are where the East Pacific Rise underlies continental rocks. So the identification of present and past intersecting regions is likely to be of great importance.

As the Atlantic and Indian Oceans originated from the break up of the Gondwanan and Laurasian continents, the fractured edges must have been subjected to oceanic ridge heating at their inception. This heating would coincide with the accumulation and migration of organic materials in these areas as the persistent subsidence afforded troughs into which organic material could be trapped and also allowed a thick accumulation of sediments to develop in them, giving rise to burial heating of older sediments. In general, direct heat flow is likely to have been most significant along the margin²³ and burial heating would be dominant within a few hundred kilometres of the margin. This subsidence was usually along pre-existing fractures where these paralleled the opening margins and the deepest subsidence took place on the continental side of fault blocks as they respond to upper mantle and lower crustal flow. Seafloor spreading also seems to be the cause of the formation²⁴ of marginal basins, such as the Japanese Sea, which also contain shallow sedimentary rocks. Thus the availability of source rocks and heating by burial and elevated heat flow implies that these regions, particularly in the West and Southwest Pacific, are likely to be areas of high hydrocarbon potential.

On the plate tectonic model igneous activity and high heat flow are absent away from plate margins, with the minor exception of kimberlite-type intrusions²¹. Deep burial heating can still occur away from plate margins as a result of upper mantle/lower crustal movements, but significant heating in such areas presumably arises mainly from exothermic chemical and biochemical reactions which may accompany diagenesis and subsequent compaction. Most of these reactions are independent of plate tectonic concepts, but some important reactions, such as between organic materials and anhydrite¹³, will occur chiefly in sediments originally deposited at low latitudes.

The final composition of the reserves reflects the nature of the source materials, the temperature and organic activity in the source, during migration and after preservation in reservoirs, and the distance between source and the reservoir. So fatty, heavy grade oils are particularly associated with deltaic conditions²² where migration has been restricted and the original organic material is often derived from brackish environments. Deltas also tend to have substantial gas associated with their high vegetal content. The variations of sulphur content within oils, or nitrogen within gas, are significant to extraction and refining techniques and prediction of the nature of the rocks through which the hydrocarbons have passed by determining their palaeolatitudinal history will lead to a closer understanding of natural refining processes and therefore to a prediction of the quality of hydrocarbon reserves. Sulphur content^{26,27}, for example, seems to correlate broadly with the presence of evaporites in the source rocks, but the final composition is probably determined largely by bacterial activity, particularly within the reservoir rocks.

Reservoirs

Various structures may trap oil and gas⁵ and plate tectonic activity is involved in each, although some links are tenuous. Stratigraphic traps, for example, are related to the detailed local palaeogeography, but the number of traps in any one region is controlled by broader considerations. For example, structural traps, such as anticlines and domes, are directly related to tectonic processes along subduction and continental collision zones. Thus the gas fields marginal to the Pyrenees owe their origin to the relative movements between Iberia and France and the Argentine fields probably formed in response to movements of the Patagonian arc⁶.

Evaporites are again significant because they lubricate tectonic movements, and afford an impervious seal for trapped hydrocarbons. Their ability to flow when the overburden exceeds some 600 m (ref. 28) means that they can create their own structural traps. The role of reefs and sand bars in evaporite formation also means that these offer potential reservoirs close to hydrocarbon sources. The margins of separating continents also provide structures, accompanying the faulting and folding movements, which may act as reservoirs.

Implications for Fuel Supplies

Concepts of continental drift and plate tectonics have a much more fundamental part to play in the evaluation of potential oil and gas reservoirs than in the mere matching of, for example, oil traps bordering the Atlantic. Physiological location of deltaic sources deposited during the past 200 m.y. can be undertaken effectively only when continental movements are taken into account and similarly the structural evolution of an area should be related to the different distribution, movements, collisions and separations of the continents. The most striking feature is that palaeo-

climatic consideration plays a vital part in the origin, migration and accumulation of hydrocarbons. Palaeomagnetic techniques, which offer a precise, economic measure of palaeolatitude, are therefore likely to be of increasing significance.

As technology advances, it should be possible to extract oil and gas from some of the extensive tar shales as well as from inhospitable yet potentially productive areas such as the Labrador Sea, Newfoundland Shelf, northern Greenland and the Canadian Arctic Islands. Nonetheless, the fact that low palaeolatitudes are so significant suggests that the southern continents which, as Gondwanaland, have mostly lain at high to intermediate palaeolatitudes during the past 400 m.y., are unlikely to be as productive as the northern hemisphere—although the size of Gondwanaland implies that some areas must have been at low latitudes for appreciable periods of time. So something like two-thirds of the world's total reserves are likely to be in the present northern hemisphere, which are already the best known areas and the regions of highest exploitation. Major reserves which are largely undetected or unexploited must still exist, for instance, off north-western Australia, the south-west and western Pacific, as well as in untapped Mesozoic-Cainozoic deltas. It is also clear that the deep oceanic parts of the world are not likely to contribute to future needs for organic fuels. It should now be possible to make a realistic estimate of the world's hydrocarbon potential and thereby evaluate the optimum development of these resources for the benefit of mankind as a whole.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Origin of Broad Emission Lines from Quasistellar Objects

THE optical spectra of QSOs exhibit very broad high-excitation emission lines. The broadest of these in the visible region for typical redshifts are the resonance lines Ly α λ 1216, CIV λ 1550 and MgII λ 2798 (ref. 1). Previous suggestions for the origin of the breadth of these lines include electron scattering and Doppler shifts due to turbulence or other mass motions in clouds of gas.

We have recently proposed a radially-streaming-proton model that gives an excellent fit to profiles of the broad H α emission lines from three different Seyfert galaxy nuclei (ref. 2 and our unpublished work). We suggest here that a similar idea will also explain the observed width of QSO emission lines. In the model, suprathermal protons slow down as they stream radially through a hydrogen gas that is at rest with respect to their source at the centre. Hydrogen emission from these protons occurs when they have slowed to speeds less than about 5,000 km s⁻¹ (about twice the speed of an electron in the first Bohr orbit), and are able to carry an electron for an appreciable fraction of the time. Thus the hydrogen emission profiles are predicted to all have overall Doppler widths at near zero intensity of about 10,000 km s⁻¹.

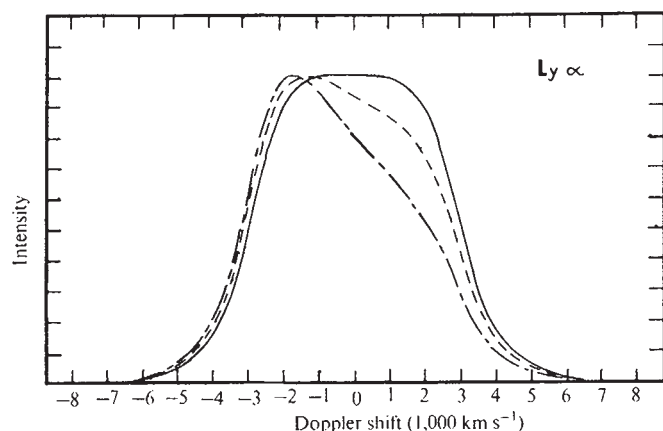


Fig. 1 Profile of Lyman alpha resulting from a radially-streaming-proton model for three different values of the transparency of the emitting region. The parameter Δ is the ratio of the photon mean free path to the radius of the emitting sphere: —, $\Delta = \infty$; ---, $\Delta = 5.0$; - · -, $\Delta = 2.0$.

In Fig. 1 we display three profiles for Ly α computed in such a model using our (unpublished) atomic cross sections. The profiles differ only in the assumed transparency of the region where the emission originates, which is the only parameter in the model that changes the profile. The overall width is deter-

mined by atomic constants; the width of the profile, measured between the two inflexion points at complete transparency, amounts to $\Delta\lambda/\lambda$ equal to 3.3 times the fine structure constant. The total emission in Ly α from the streamers is computed to vary from about 600 photons per proton when the gas is neutral, to about 6 photons per proton at 90% ionization for the gas.

We have made preliminary calculations of profiles for CIV λ 1550, again assuming that the carbon nuclei are decelerating as they stream radially through a hydrogen gas. The necessary atomic cross sections are known with less certainty than for the Ly α case so the width is less well defined. It is clear, however, that the shape of the CIV resonance line should resemble the Ly α profile, especially in the wings, but be broader by a factor of approximately the square root of the ratio of the ionization potentials of CIV and hydrogen (about 2.2). Because it is primarily the speed of the outermost electron of the ion that determines the behaviour of the atomic cross sections for charge transfer and ionization, the widths of profiles of resonance lines of other ions can be predicted by the same scaling procedure.

There seem to be very few published high-resolution profiles of the broad emission lines from quasistellar objects, but those we have seen³ seem to agree with what we have calculated. Low resolution observations have been made on several QSOs by Oke^{4,5}. A detailed comparison of calculated and observed profiles is not meaningful at this resolution (about 50 to 100 Å), but it is interesting that the hydrogen lines are typically quite broad with a full-width at zero intensity on the order of 10,000 km s⁻¹, and the resonance line of CIV seems to be broader still. The data for 3C273 are consistent with half-half widths of the Balmer lines of about 2,500 km s⁻¹, which is what one expects from our model. What is needed for a good test is a determination of these profiles with a resolution of a few angstroms.

Because of apparent association of QSOs of different redshifts, the failure of QSOs to conform to the same Hubble law as known galaxies and the different redshifts of emission and absorption lines in the same QSO, it has been suggested that new physical laws may be required to describe them (ref. 6 and G. Burbidge, unpublished). If the profiles we have computed agree with QSO emission profiles as well as they do with the hydrogen emission profiles of Seyfert galaxies, then it will be strong evidence that at least the fine structure constant must have the same value in a QSO as it does in the Solar System.

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Palaeomagnetic Evidence for Rotation of Sardinia during the Early Miocene

GEOLOGICAL and palaeomagnetic evidence indicates that after the Permian the Corso-Sardinian plate rotated counter-clockwise with respect to stable (extra-Alpine) Europe¹⁻⁶. Palaeomagnetic directions from Late Oligocene/Early Miocene rocks can be interpreted as indications for a Late Tertiary rotation^{5,7}. Here we present additional palaeomagnetic data from volcanic rocks and tuffites of Tertiary age, collected near the village of Castelsardo on the northern coast of Sardinia (Fig. 1).

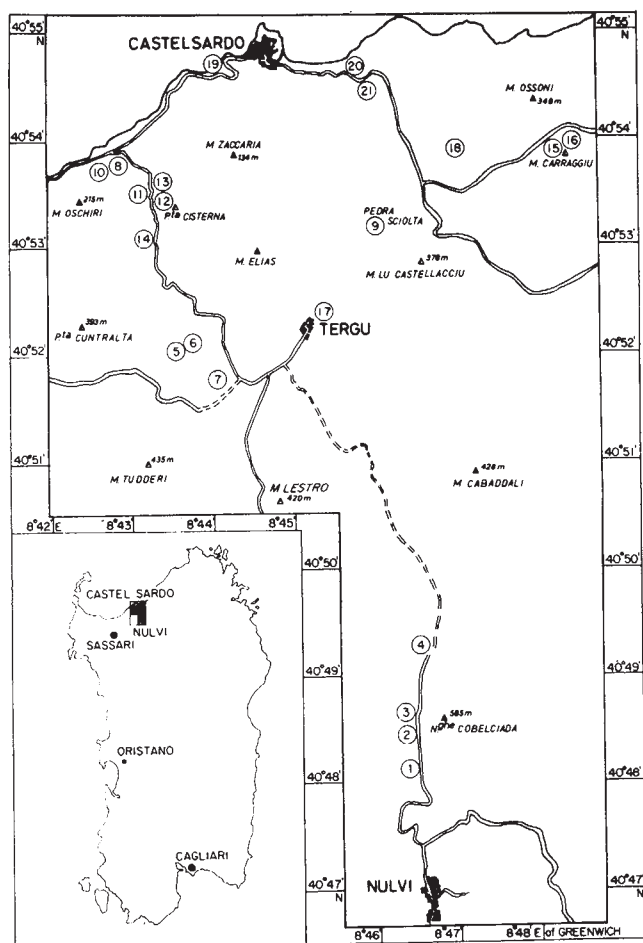


Fig. 1 Map of the Castelsardo area with sampling sites indicated.

The volcanic rocks of northern Sardinia consist of andesites, trachytes and liparites. Tuffs, tuffites and sediments are intercalated. The exposures are discontinuous, the sediments show rapid facies transitions, and block faulting hampers detailed correlation of the volcanic rocks. Near Castelsardo, however, it is possible to map in detail a number of stratigraphical units, from which oriented hand samples were collected. The geological relationships of these units are depicted schematically in Fig. 2. The two principal andesite bodies in the area (indicated as α on the geological map of Moretti *et al.*⁸) underlie all other sampled units, the oldest of which are Aquitanian. Comparison with other volcanic units outside the Castelsardo area suggests that the andesites are not older than Middle Oligocene⁹. Their age must be similar to that of the Alghero trachyandesites; a contemporaneity supported by similar palaeomagnetic directions.

The overlying lacustrine sediments, tuffs and tuffites are dated as Aquitanian/Langhian on the basis of contained

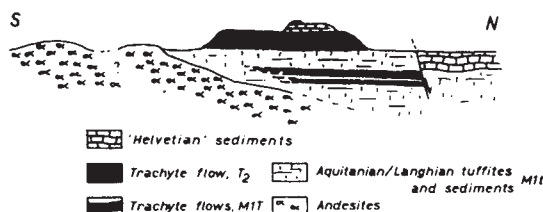


Fig. 2 Schematic stratigraphical section of the sedimentary and volcanic rocks which were sampled for this study.

fossils (refs. 10, 11 and M. L. Colalongo, personal communication). Intercalated in the sediments and tuffites (M1t) are two flows of trachyte (M1T). Samples from two sites within M1t and from seven sites within M1T yielded similar palaeomagnetic results. Small local andesite plugs and intercalations also occur, but they have not been sampled extensively because of uncertain stratigraphical relationships.

There is a thick trachyte flow capping the sequence of M1t sediments, tuffs and M1T flows. Southeast of Castelsardo this flow is usually denoted as T₂, southwest of Castelsardo T₂ δ and T₃ are also used. Sampling was restricted to the exposures denoted as T₂, because the geological correlation between the T₂ exposures is unambiguous and because its age is better established: for instance 2 km southeast of Castelsardo, the flow is overlain by marine sediments of Serravallian (Helvetian auct.) age¹¹.

The natural remanent magnetizations of ninety-five hand samples from twenty-one sites have been studied in the

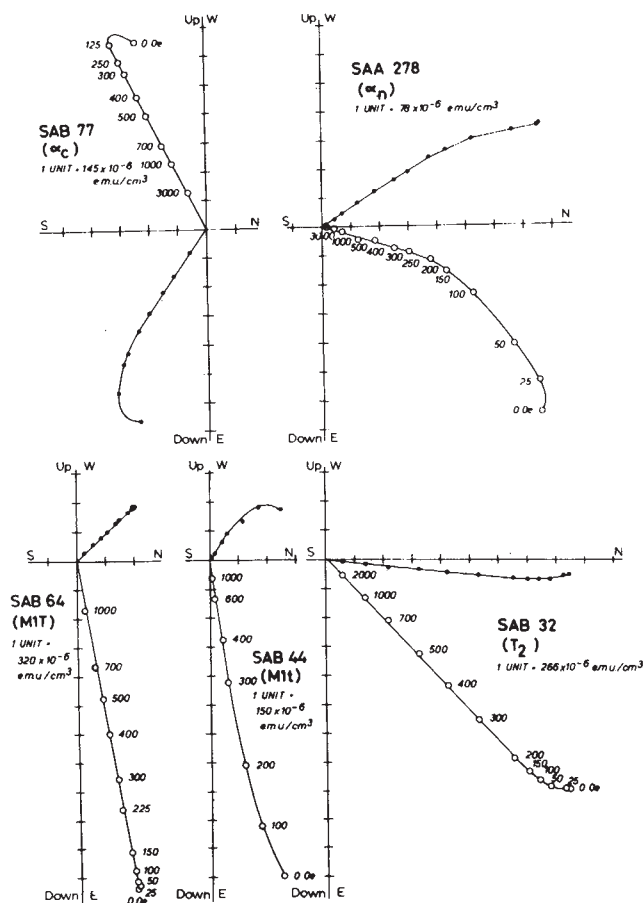


Fig. 3 Demagnetization diagrams obtained from samples from the five stratigraphical units investigated. Plotted points represent the end points of the magnetization vector—in orthogonal projection—during progressive AF demagnetization. Full symbols represent projections on the horizontal plane; open symbols represent projections on the N-S vertical plane.

Palaeomagnetic Laboratory of the State University of Utrecht. The intensities of the magnetizations were of the order of 200×10^{-6} to $9,000 \times 10^{-6}$ emu cm $^{-3}$; the Q -values varied between 0.4 and 30, but were generally higher than 1.0. Step-wise demagnetization in alternating fields of up to 3,000 Oersted generally revealed that secondary magnetizations play no important role and that they can be easily removed in the lower alternating fields (Fig. 3). Fields of 3,000 Oersted usually also completely eliminated the characteristic magnetizations. The direction of the characteristic magnetization was determined from the final straight trajectory of the demagnetization diagrams (for example, the straight paths between 300 and 1,000 Oersted in Fig. 3). The "within-site" scatter of the directions of magnetization was very small and generally decreased even more on demagnetization.

In Fig. 4 we plot the mean site directions of these stratigraphical units (with respect to the original horizontal) together with those previously published for Tertiary rocks from Sardinia. Both the inclination and the declination values show a large variability. The variability in declinations is remarkable because here there seems to be a consistent trend: the oldest rocks show the most westerly declinations (Fig. 4a), younger rocks a NNW declination and the youngest rocks (flow T_2 and the two Plio-Pleistocene basaltic formations β_1 and β_2) possess a direction close to due north (Fig. 4b).

This change in declination could be the result of a long lasting Late Oligocene–Early Miocene excursion of the geomagnetic dipole field, but such an event has not been demonstrated for this period. We conclude, therefore, that it is more likely that the change in declination represents a

Table 1 Summary of the Tertiary Palaeomagnetic Data from Sardinia

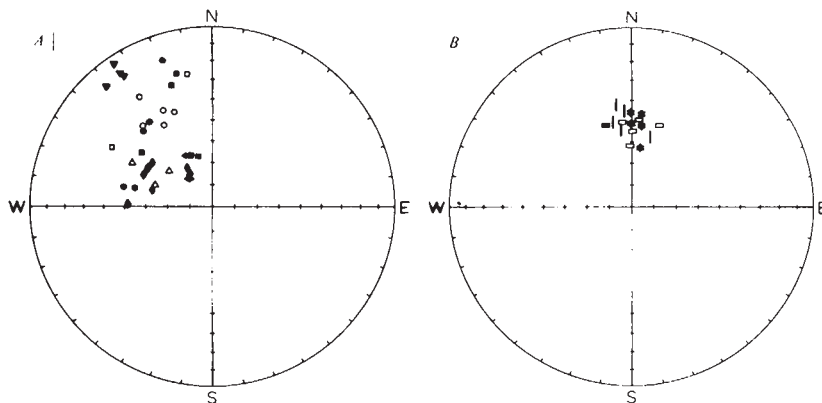
Age	Formation	Map symbol ⁸	Site No. Fig. 1	Decl. Incl. per site *	N (n) †	K	Mean direction of magnetization α_{95}	Decl.	Incl.	Pole position and (dp, dm)
Post-Tortonian, mainly Pliocene and Quaternary	Basalts near Logudoro ⁷	β_1		14.5 + 56.5 353.5 + 52.5 347.5 + 49.5	3 (8)	80	14°	357.5	+53.5	83 N, 154 W (13.5; 19.5)
	Basalts near Logudoro ⁷	β_2		360 + 46.5 355.5 + 46.5 351 + 41	3 (9)	321	7°	355.5	+44.5	75 N, 155.5 W (5.5; 8.5)
	Basalts near Orosei ¹³	?		181 – 62 199 – 50 174 – 51 180 – 55 181 – 55	5 (34)	120	7°	183	–55	84.5 N, 162.5 E (7; 10)
	Basalts from Campidano ¹³	?		183 – 51	1 (9)	—	—	183	–51	81.5 N, 171 E (9; 13.5)
	Basalts near Campeda ¹³	$\beta_1 + \beta_2$		342 + 50	1 (5)	—	—	342	+50	72.5 N, 108.5 W (9; 13.5)
Middle/Late Miocene	Castelsardo trachyte (our work)	T_2	17 18 19 20 21	359.5 + 51 359 + 46 6.5 + 52 5.5 + 46.5 7.5 + 62.5	5 (25)	131	6.5°	3.5	+51.5	81 N, 170 E (6; 9)
	Aquitania/Langhian tuffites of Castelsardo (our work)	M1t	8 9	334 + 64 321.5 + 73.5	2 (9)	120	23°	329	+69	66 N, 42 W (33; 39)
Early Miocene	Aquitania/Langhian trachyte flows near Castelsardo (our work)	M1t	10 11 12 13 14 15 16	324 + 72.5 327.5 + 69 287 + 62 301 + 56 272 + 51.5 295.5 + 55.5 305.5 + 56	7 (32)	46	9°	298.5	+61.5	45 N, 57.5 W (10.5; 14)
	Andesite body, south of Castelsardo (between Nulvi and Tergu (our work)	α_n	1 2 3 4	325.5 + 11.5 318.5 + 12.5 325.5 + 5 326 + 14	4 (17)	234	6°	324	+11	42.5 N, 119 W (3; 6)
Oligocene–Early Miocene	Andesite of Contralta near Castelsardo (our work)	α_c	5 6 7	130.5 – 65 110 – 62.5 119 – 48.5	3 (11)	64	15.5°	119.5	–59	45 N, 62 W (17.5; 23)
	Alghero trachyandesites ⁵	T_1		148 – 47 317 + 43 324.5 + 42.5 341 + 16 153 – 40 162.5 – 43 146 – 28 138.5 – 43 284 + 54.5 283 + 49	10 (59)	20	11°	322	+42	54 N, 95 W (12.5; 17)
	Pre-Helvetian volcanics near Logudoro ⁷	α (α_1 ?)		344 + 66.5 120.5 – 35.5	7 (28)	12	18°	332.5	+43.5	62.5 N, 106.5 W (14; 22.5)
		α (α_2 ?)		307 + 49						
		T_2		341.5 + 29.5						
		T_3		345 + 24.5						
		T_3' (2)		337 + 64.5						
		α_3		169 – 25.5						

Geographical data according to the 1 : 100,000 sheets of the Geological Map of Italy.

* After demagnetization (500–3,000 Oersted) and after correction for the dip of the beds.

† N, Number of sites or flows, used in the statistical analysis; n, the number of samples investigated.

Fig. 4 Equal area projections of the site-mean directions of magnetization of the Tertiary rocks from Sardinia (see also Table 1). *a*, The Oligocene/Lower Miocene rocks; *b*, the Middle Miocene and younger rocks. All symbols are projections of the impingement points on the lower hemisphere; open symbols indicate palaeomagnetic directions which had to be inverted to impinge the lower hemisphere (reversed polarity), full symbols indicate directions with normal polarity. □, ■, Ref. 7; ○, ●, ref. 5; △, ▲, α_c andesite; ▼, ▽, α_n andesite; ◆, ◇, Mlt sediments and tuffs; ♦, ♦, Mlt flows; ★, T₂ flow; □, ■, ref. 13; ▨, ▩, ref. 7.



counterclockwise rotation of Sardinia over an angle of approximately 50°. Because the declinations of the tuffites of Mlt are intermediate, it may be argued that the rotation of Sardinia actually occurred during the sedimentation of the Aquitanian/Langhian (Early Miocene) rock sequence of Castelsardo.

In summary, the lower time limit of the rotation, presumed on palaeomagnetic grounds, seems now rather well established as Late Oligocene/Early Miocene. The Castelsardo research suggests that the minimum age of the upper limit is indicated by the palaeomagnetic direction of unit T₂. The pre-Saravallian age of these rocks could imply that the rotation was completed before the Middle Miocene, although this is at variance with the hypothesis that the crustal block of Sardinia had not completed its rotation until the Pliocene¹³.

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Climatic Implications of Total Gas Content in Ice at Camp Century

WHEN firn turns into ice through densification and recrystallization, the volume of atmospheric gas trapped as the pores close off depends in particular on the atmospheric pressure. The idea of a correlation between the total gas content and the site formation altitude of high polar ice has thus been suggested¹. This concept received new consideration when observations of climatic changes based on the isotopic composition of ice sampled along high polar ice cores were carried out²⁻⁴, because the variations in measured isotopic content depend not only on general climatic changes (especially temperature changes) but also on different parameters among which the site formation altitude of the ice could play an important part.

Here we present the results of twenty-five total gas content determinations (Fig. 1) on apparently unfractured samples taken from the 1,400 m deep core drilling carried out at Camp Century on the North Greenland ice sheet⁵. A careful study of the isotopic composition of this ice enabled the observation of climatic variations over more than 100,000 yr (ref. 6). The total gas content of the ice was measured volumetrically in a MacLeod gauge once the sample has been melted in vacuum and the gas phase completely extracted with water vapour trapping.

The total gas content V reduced to STP and 1 g of ice can be theoretically defined by

$$V = (1/\gamma_c - 1/\gamma_i) T_0 P_c / T_c P_0$$

γ_c and γ_i being respectively the pore close-off density and the pure ice density at temperature T_c , T_0 the standard temperature (273.16 K), T_c and P_c the *in situ* temperature (K) and the average pressure of the gas occlusions at pore close-off, and P_0 the standard pressure.

If the climatic conditions of the formation site (temperature and pressure) are known, the measurement of V permits a calculation of γ_c . Determinations for Byrd Station (Antarctica) and Camp Century show that the total gas content within the ice remains unchanged and that the close-off densities under present climatic conditions are practically identical (0.822 ± 0.003 and 0.823 ± 0.004) and in excellent agreement with the only other precise value that has been published^{7,8}: 0.824 ± 0.004 for site 2, Greenland.

Nevertheless, in the case of Camp Century, a sudden and significant decrease in the total gas content is observed below a depth of approximately 1,100 m. This discontinuity corre-

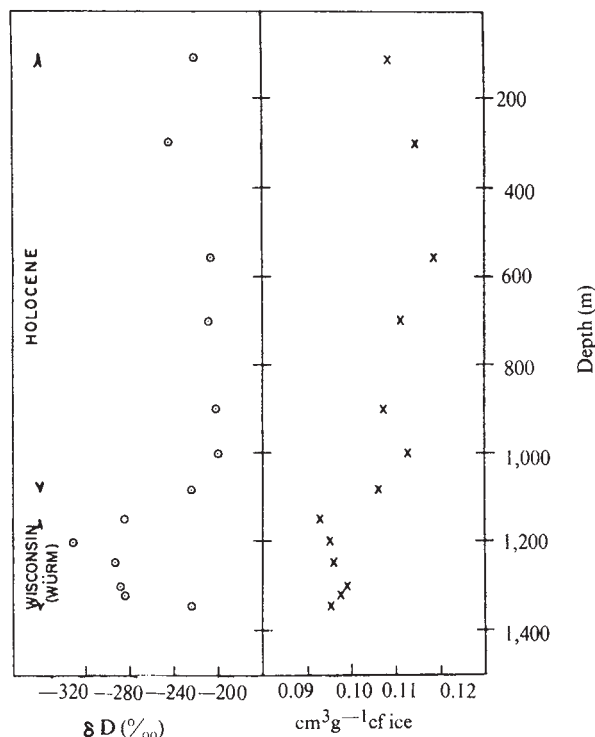


Fig. 1 Deuterium and total gas content against depth at Camp Century. Determinations of total gas content are averaged for close proximity samples.

sponds to the end of the last glaciation as is shown by the isotopic composition profile (Fig. 1) which agrees with the detailed study published by Dansgaard and others⁶. The total gas content of Wisconsin (Würm) ice is on average 12.5% lower than that of the Holocene; a decrease in temperature should have led to higher values. This decrease in the total gas content could be due to a higher close-off density during the last glaciation; alternatively, the site formation altitude of Wisconsin ice was higher than that of the present Camp Century.

Assuming γ_c has remained constant, this altitude difference would have to be about 1,300 m to account for the observed results if we use a temperature-elevation gradient of $0.9^\circ \text{C}/100 \text{ m}$ (ref. 9) and a pressure-elevation gradient of $7 \text{ mm Hg}/100 \text{ m}$. Any general decrease in temperature linked to the last glaciation would make this difference even more pronounced. According to present ice surface elevation contours in Greenland, ice satisfying this altitude condition would have been formed at a distance from Camp Century (of the order of 800 km) which is not compatible with the present surface ice velocity (a few m per year at Camp Century). Consequently, this suggests a significant increase in the thickness of the ice sheet in this part of Greenland during the last glaciation.

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Shear Elastic Constant C_{44} and Phase Transformation of KCl

THE shear elastic constant C_{44} for KCl, KF and the rubidium halides is of special interest because it decreases with pressure. Born¹ and Misra² pointed out that a vanishing shear elastic constant is a sufficient condition for a phase transformation. This suggestion has been elaborated by Anderson and Demarest³ and by Thomsen⁴ to postulate that the phase transformation from the NaCl to CsCl structure at high pressure may be associated with the vanishing of C_{44} . If borne out by experiment, this suggestion would be of considerable physical interest. Based on values of the first pressure derivative of C_{44} for the halides, measured at relatively low pressure, Demarest⁵ extrapolated C_{44} to high pressure and showed that the phase transitions actually take place at much lower pressures than those predicted by the vanishing of C_{44} . Such extrapolation, however, does not rule out the possibility of a large downward curvature in the C_{44}/P relation at higher pressures, especially at pressures near the phase transformation leading to a vanishing C_{44} . Thus direct measurement of C_{44} up to the pressure of phase transformation appears necessary for a test of the hypothesis. I present here experimental results for ultrasonic determination of C_{44} for a KCl single crystal up to 20 kbar at which pressure it transforms from its NaCl structure to the CsCl structure.

The high pressure apparatus is of the Bridgman type^{6,7}, capable of generating hydrostatic pressure up to 30 kbar. The pressure-transmitting medium was 2-methylbutane. Pressures up to 4 kbar were measured by a Heise gauge calibrated against a free piston gauge. Above 4 kbar, a manganin resistance coil calibrated against the Heise gauge up to 4 kbar was used to monitor pressure. The accuracy in pressure determination is 0.1% up to 4 kbar and 0.5% at higher pressures.

The single crystal was of optical quality; the two surfaces parallel to (110) were polished flat. Two a.c.-cut quartz

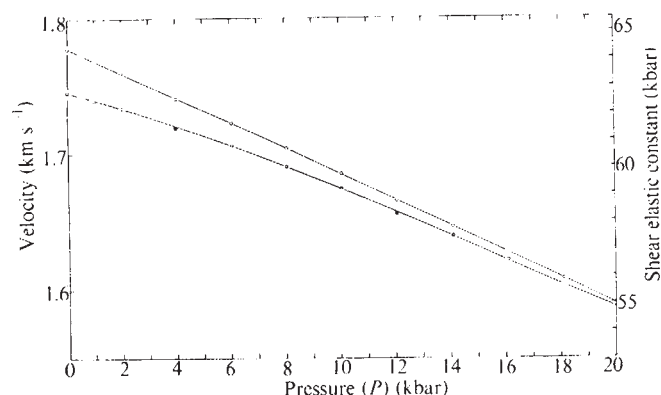


Fig. 1 Velocity (○) and shear elastic constant (◻) of KCl against pressure.

transducers of 20 MHz were mounted on these surfaces to generate shear vibrations in the (100) direction. The conventional "pulse transit" technique⁸ was used to determine the transit time of ultrasonic pulses through the specimen. The procedure for calculating velocity and C_{44} from the measured transit time is elementary; Murnaghan's equation of state, together with the elastic constants determined by Bartels and Schuele⁹, were used to estimate sample density and the change of its dimension under pressure. Accuracy in the velocities is estimated to be 1%; relative precision among the measurements is better than 0.1%.

Both the velocities and the corresponding values of C_{44} are plotted against pressure in Fig. 1. The pressure derivative of C_{44} at 1 atm is found to be -0.31 , in good agreement with the determination of Bartels and Schuele⁹. A slight downward curvature appears in the C_{44}/P relation, but this curvature does not become great enough to bring C_{44} near to zero. The relatively large uncertainty in pressure determinations above 4 kbar does not allow an accurate determination of the magnitude of this curvature. I conclude that the phase transformation of KCl from its NaCl to the CsCl structure is not associated with the vanishing of C_{44} .

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BIOLOGICAL SCIENCES

Enhanced Utilization of Brain Acetylcholine during Morphine Withdrawal in the Rat

MORPHINE prevents the release of acetylcholine (ACh) from cholinergic neurones¹⁻⁹ when the neurones are stimulated at a low but not at a high frequency. Thus a special mechanism for neurotransmitter release at low frequencies seems to be involved¹⁰. Although the relationship of this effect of morphine to analgesia is unclear, it may be important to the sedative actions of morphine as well as to the phenomenon of physical dependence. Some aspects of the morphine withdrawal syndrome have a cholinergic component¹¹⁻¹³. Morphine reduces the release of brain ACh^{1-9,14,15} so it is to be expected that its turnover is reduced in acutely narcotized animals, and enhanced during withdrawal of chronic morphine dependent animals.

Direct measurements of ACh turnover have not been made in morphine dependent animals because of methodological complexities. Large and Milton^{16,17}, however, using the cholinesterase inhibitor, physostigmine, provided indirect evidence for enhanced ACh turnover during morphine withdrawal in the rat. This report describes data which are compatible with their conclusion, but which were obtained

using two different inhibitors of ACh synthesis. One of these, hemicholinium-3 bromide (HC-3), presumably acts by preventing choline transport across various cellular membranes¹⁸⁻²¹. The other, acetylseco hemicholinium-3 bromide (acetylseco HC-3), is an inhibitor of choline acetyltransferase²². Intraventricular (i.v.t.) injection of these compounds reduces total brain ACh. This effect is reversed by morphine and related narcotic agonists and narcotic antagonists²³⁻²⁵. The technique is not a direct measure of brain ACh turnover. It is an acceptable, indirect approach to the study of ACh utilization because (a) the expected decrease in brain ACh was observed, (b) the incorporation of ¹⁴C-choline into ¹⁴C-ACh was clearly reduced (Domino *et al.*, unpublished results), and (c) data obtained with pentobarbital using this method are in complete agreement with those reported for pentobarbital using incorporation of ¹⁴C-choline into ACh as a measure of turnover^{26,27}.

Male Holtzman rats (20-30 days old) were housed in group cages in an artificially illuminated room maintained at $24 \pm 2^\circ \text{C}$. All animals were kept on an automatic 7.30 a.m. to 12.00 p.m. light and 12.00 p.m. to 7.30 a.m. dark cycle. Food and water were freely available. Morphine was administered twice daily at approximately 8.00 a.m. and 8.00 p.m. on the following schedule for the 2 week series: day 1, 10; day 2, 20; day 3, 30; day 4, 50; day 5, 60; day 6, 80; day 7, 100; day 8, 110; day 9, 120; day 10, 130; day 11, 150; day 12, 160; day 13, 180 and day 14, 200 mg kg⁻¹.

Animals given morphine for 8 weeks were on the following twice daily schedule: day 1, 20; day 2, 25; day 3, 30; day 4, 35; day 5, 45; day 6, 50; day 7, 55; day 8, 60; day 9, 65; day 10, 70; day 11, 75; day 12, 80; day 13, 85; day 14, 90; day 15, 95; day 16, 100, and 100 mg kg⁻¹ for the remaining 6 weeks. Groups of at least twelve animals were run initially. There were six or more surviving animals per group. All injections were given intraperitoneally (i.p.) except in the animals receiving morphine for 8 weeks, where occasional subcutaneous (s.c.) injections were given to reduce mortality. Control animals were given 0.9% NaCl. After chronic administration of morphine for 15 or 57 days, animals were given, in the morning at the regularly scheduled time, a narcotic or saline, i.p., and HC-3 and/or acetylseco HC-3, i.v.t., and 30 min later killed by decapitation. All animals were given diethyl ether-air anaesthesia for the i.v.t. injections and allowed to recover. Animals were killed after 30 min because the rate of depletion of ACh was relatively linear at this time. ACh depletion was almost maximal with a survival rate of approximately 80%²³⁻²⁵. Total ACh was extracted from brain tissue (minus cerebellum) with the acid alcohol method of Stone²⁸ and bioassayed using the frog rectus abdominis method as described by Feldberg²⁹. Hydrolysed tissue extracts to which ACh standard was added were used to account for the presence of sensitizing factors. All drugs were given in conveniently available salts and dosage calculated as base content. Because HC-3 and acetylseco HC-3

Table 1 Effects of Various Control Procedures, Morphine and Nalorphine on Rat Brain Acetylcholine after Acute Drug Administration

Treatment	Dose of narcotic (mg/kg)	No.	Brain ACh mean $\pm$ s.e. nmol g ⁻¹	P value *
NaCl, 0.9%	—	8	18.7 $\pm$ 0.4	—
Nothing	—	8	18.1 $\pm$ 1.3	NS
NaBr i.v.t.	—	8	17.4 $\pm$ 0.9	NS
HC-3, 20 μ g	—	11	9.7 $\pm$ 0.4	<0.001
Acetylseco HC-3, 5 μ g	—	8	10.6 $\pm$ 0.4	<0.001
Morphine	10	8	19.1 $\pm$ 0.9	NS
Morphine	200	8	22.1 $\pm$ 0.5	<0.01
Morphine + 20 μ g HC-3	10	12	17.2 $\pm$ 1.0	NS
Nalorphine	20	8	18.8 $\pm$ 0.7	NS
Nalorphine + 20 μ g HC-3	10	8	8.2 $\pm$ 0.1	<0.001

* Group comparison Student "t" test to post-ether 0.9% saline treated rats.

Table 2 Effects of Morphine Withdrawal on Brain Acetylcholine after Chronic Administration of Morphine for 2 and 8 Weeks

Drug	Dose of drug (mg kg ⁻¹)	No.	Brain ACh mean ± s.e. (nmol g ⁻¹)	<i>P</i> value *	
				A	B
2 week tolerant					
Morphine	200	9	16.6 ± 0.4	NS	
Nalorphine	10	10	14.6 ± 0.5	< 0.01	
48 h withdrawn	0	9	14.4 ± 0.4	< 0.01	
Morphine + 20 µg HC-3	200	6	11.4 ± 0.3	< 0.001	NS
Nalorphine + 20 µg HC-3	10	8	7.9 ± 0.2	< 0.001	NS
48 h withdrawn + 20 µg HC-3	0	7	8.0 ± 0.3	< 0.001	NS
Morphine + 5 µg acetylseco HC-3	200	8	14.7 ± 0.3	< 0.01	< 0.001
Nalorphine + 5 µg acetylseco HC-3	10	10	7.8 ± 0.1	< 0.001	< 0.001
48 h withdrawn + 5 µg acetylseco HC-3	0	10	8.0 ± 0.2	< 0.001	< 0.001
8 week tolerant					
NaCl, 0.9%	0	7	16.8 ± 0.3	NS	
Nalorphine	50	8	15.8 ± 0.5	NS	
48 h withdrawn	0	8	11.8 ± 0.5	< 0.001	
Nalorphine—10 min until death	50	7	14.4 ± 0.4	< 0.01	

* Group comparison Student "t" test. In column A treated rats are compared with acute 0.9% NaCl controls (data in Table 1) except for 8 week morphine tolerant animals which are compared with 8 weeks of 0.9% NaCl. In column B the values of brain ACh after narcotic plus ACh depletor are compared with the values after ACh depletor alone taken from Table 1.

were available as the Br salt, NaBr was used as a further control.

The mean brain ACh ± s.e. in nmol g⁻¹ wet weight for various control procedures and treatments are given in Table 1. Control steady state brain ACh values for naive animals and animals treated with 0.9% NaCl were not significantly different. Both HC-3 and acetylseco HC-3 given i.v.t. reduced brain ACh. Morphine but not nalorphine prevented the reduction of brain ACh by HC-3. In doses of 10 mg kg⁻¹, i.p., neither affected steady state brain ACh.

The effects of giving morphine for either 2 or 8 weeks twice daily differed in several important ways from those obtained with single, acute injections. As shown in Tables 1 and 2, a single large dose of morphine caused a significant increase ($P < 0.01$) in steady state brain ACh of normal animals, but not in 2 week tolerant rats. Nalorphine and 48 h abrupt withdrawal both significantly ($P < 0.01$) reduced steady state brain ACh. In 2 week tolerant rats, 200 mg kg⁻¹ of morphine did not prevent depletion of brain ACh by HC-3 or acetylseco HC-3 as much as 10 mg kg⁻¹ in non-tolerant rats did. Brain ACh was depleted even more during nalorphine and 48 h morphine withdrawal by 5 µg i.v.t. acetylseco HC-3 than in non-tolerant rats. In 8 week tolerant rats 48 h withdrawal and 10 min after nalorphine, there was a significant ($P < 0.001$) decrease in steady state brain ACh. Although these data on steady state brain ACh disagree with those of Large and Milton^{16,17}, the findings of decreased utilization during acute morphine and enhanced utilization during withdrawal are in complete agreement. Different strains or the sex of the Wistar rats (female) may respond differently to enhanced brain ACh turnover, some showing an increase in steady state ACh and others a decrease. The interpretation of enhanced ACh turnover during morphine withdrawal using 5 µg i.v.t. acetylseco HC-3 depends on what comparison is made. In Table 1, acetylseco HC-3 reduced the ACh level from 18.7 (or 17.4) to 10.6, a mean reduction of 8.1 (or 6.8) nmol g⁻¹. In Table 2, this inhibitor reduced the ACh level from 14.6 (in the nalorphine group) to 7.8, a reduction of 6.8; and from 14.4 (48 h withdrawal) to 8.0, a reduction of 6.4 nmol/g.

The conclusion that ACh turnover is enhanced during morphine withdrawal is valid only if it is assumed that a comparison can be made between all groups and naive controls. In any event, it is quite clear that cholinergic brain function is altered by morphine in normal, tolerant and withdrawing animals. Any theory of morphine action must take into account these dramatic changes in brain ACh and hopefully point the way to more effective treatments of narcotic dependency.

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Failure of "Dystrophic" Neurones to Support Functional Regeneration of Normal or Dystrophic Muscle in Culture

THE traditional concept that muscular dystrophy is a "primary degenerative myopathy"¹ has recently been challenged, and the suggestion put forward that the disease may in fact have a neural basis. This has been based on several independent investigations, along different lines. Dubowitz² observed "dystrophy-like" changes in the histochemical preparations of experimentally reinnervated muscle, and postulated that muscular dystrophy might be caused by an aberration of the normal controlling influence of the nervous system on muscle. In a series of electrophysiological studies in Duchenne and other forms of muscular dystrophy, McComas *et al.*³⁻⁵ demonstrated a marked reduction in the number of motor units. Similarly, in the dystrophic mouse it was found that many muscle fibres were functionally denervated⁶ and that there were fewer motor units, which were still of normal size⁷.

In transplantation studies on dystrophic mice⁸ and hamsters⁹, it was observed that either normal or dystrophic muscle transplanted into a dystrophic host failed to regenerate, whereas normal and dystrophic muscle transplanted into a normal host did regenerate, the normal muscle being morphologically normal and the dystrophic regenerate dystrophic in appearance. Salafsky¹⁰ also obtained no regeneration in the dystrophic mouse, but found that dystrophic or normal muscle transplanted into a normal host developed normal electrophysiological twitch characteristics after regeneration.

When grown in isolation in tissue culture, dystrophic human muscle had the same regenerative capacity and morphological and histochemical characteristics as normal muscle^{11,12}, suggesting that some additional influence *in vivo* caused the dystrophic change.

The evidence is still essentially circumstantial, and direct proof of an abnormal neural influence is still lacking. Thus, the possibility of humoral rather than neural influences affecting the regeneration of transplanted muscle in the intact animal has not been ruled out, and the electrophysiological studies

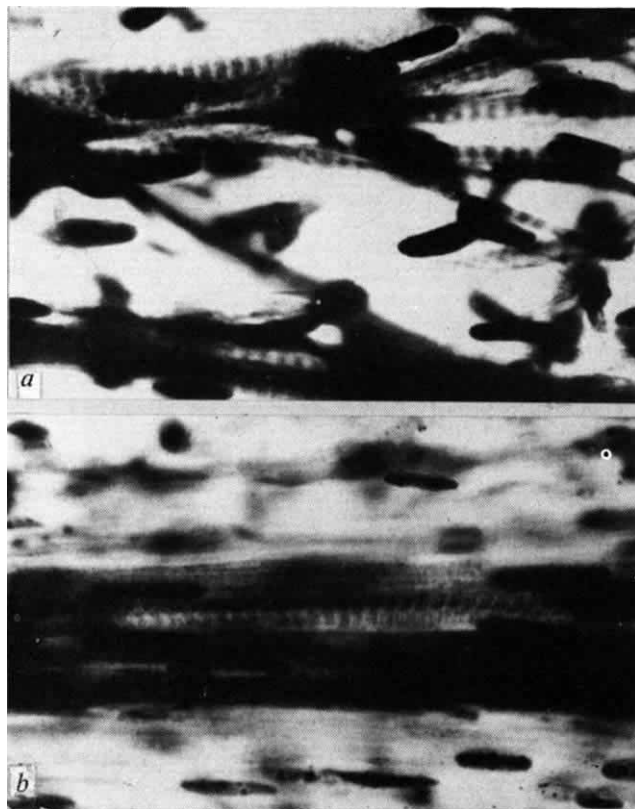


Fig. 2 Regenerated muscle fibres with well developed cross-striations, peripherally located nuclei, and spontaneous synchronized contractions. *a*, Normal muscle coupled with normal cord; 44 d *in vitro*; *b*, dystrophic muscle coupled with normal spinal cord; 25 d *in vitro*. Holmes's silver impregnation, $\times 800$.

cannot prove that the decrease in motor units is actually the primary lesion rather than an associated or even sequential pathological process.

The tissue culture of nerve and muscle in combination has advantages over other experimental models in the study of the pathogenesis of muscular dystrophy. The system is versatile, as it is possible to grow nerve and muscle tissues of different species together and obtain functional innervation without tissue rejection¹³. The selective effects of the nerve and/or muscle can be clearly defined, as the cultures are grown away from humoral influences.

In our experiments, four series of cultures were established: normal nerve/normal muscle (ten cultures); normal nerve/dystrophic muscle (twenty-one cultures); dystrophic nerve/normal muscle (six cultures); dystrophic nerve/dystrophic muscle (seven cultures). Cultures were grown¹⁴ on collagen-coated coverslips in Maximow double coverslip assemblies at 34°–35° C in the lying-drop position; twice a week they were washed in balanced salt solution and fed a fresh drop of medium (33% human placental cord serum, 10% chick embryo extract, 50% Eagle's MEM with 0.01 HEPES, 600 mg% glucose, and 1.3 $\mu\text{g ml}^{-1}$ achromycin).

Nerve explants consisted of cross-sections of spinal cord containing one pair of ganglia, dissected from 14 to 15-d-old embryonic mice, care being taken to keep the meninges intact. Five days after explantation of the spinal cord, bundles of twelve to twenty-five muscle fibres were teased from the tibialis anterior muscle of adult mice, and positioned around the ventral side of the cord. Muscle was taken from normal mice (Swiss white) and from homozygous dystrophic mice of both the Bar Harbour strain (129/reJ-dy) and the allelic form (C57Bl/6J-dy²¹)¹⁵. The clinical symptoms and histological changes are similar in both forms of dystrophy, but are less severe and of slower progression in the C57 mice¹⁵, and homozygotes for this strain are able to mate. Normal and

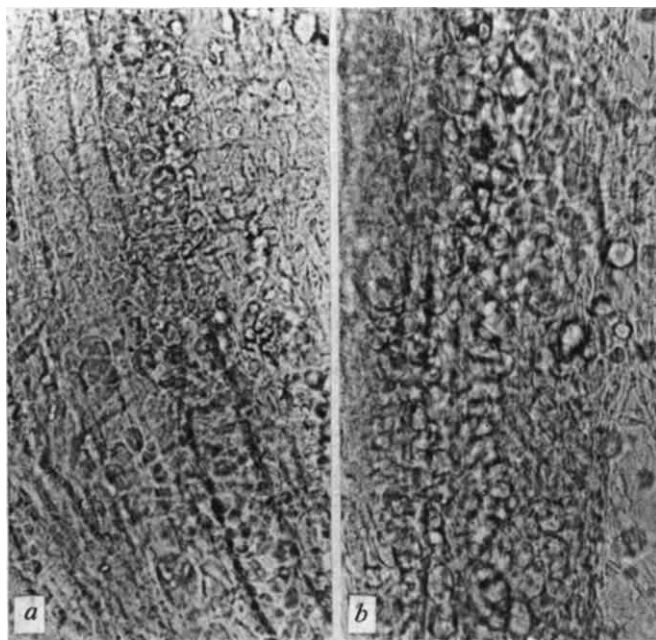


Fig. 1 Cellular regenerative phase of muscle after contact with cells from the spinal cord (to the right of the muscle). *a*, Dystrophic muscle coupled with normal cord; muscle 8 d *in vitro*; *b*, dystrophic muscle coupled with dystrophic cord; muscle 7 d *in vitro*. Unstained living cultures, $\times 160$.

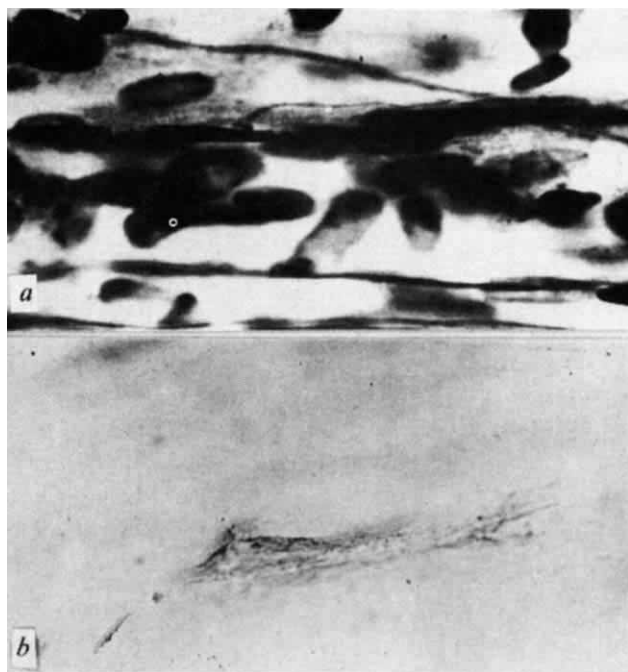


Fig. 3 *a*, Nerve ending on regenerated muscle fibre. Dystrophic muscle coupled with normal spinal cord; 28 d *in vitro*. Holmes's silver impregnation, $\times 800$. *b*, Localization of acetylcholinesterase activity at endplate on regenerated muscle fibre. Normal muscle coupled with normal spinal cord; 30 d *in vitro*. Acetylcholinesterase, $\times 800$.

dystrophic (homozygous) embryos were of the Swiss white and C57Bl/6J-dy^{2J} strains respectively.

Regeneration of the muscle was stimulated by contact with cells from the spinal cord. This occurred in all four combinations of normal and dystrophic cord and muscle (Fig. 1). The subsequent course, however, showed a striking difference between normal and dystrophic cord.

If contacted by ventral root neurites from normal spinal cord, the regenerated cells of both normal and dystrophic muscle then fused to form new muscle fibres (Fig. 2), with well developed cross-striations and peripherally located nuclei. These showed spontaneous synchronized contractions of groups of fibres. Muscle remained striated with good contractile activity throughout the time in culture (7 weeks). No differences in regeneration or survival could be detected between normal and dystrophic muscle innervated by normal spinal cord, except that there appeared to be slightly greater and quicker regeneration in dystrophic muscle. The formation of neuromuscular junctions was established on morphological (silver impregnation) and histochemical (acetylcholinesterase activity) grounds (Fig. 3*a* and *b*).

The behaviour of muscle coupled with "dystrophic" spinal cord was in striking contrast. The initial cellular regenerative response (Fig. 1*b*) was followed in nearly all cases (ten out of thirteen) by degeneration and disappearance of all muscle elements, leaving only strands of connective tissue. In only three cultures were myotubes formed (Fig. 4), some of which showed fibrillatory, labile, and asynchronous contractions. No cultures showed synchronized contractions of groups of muscle fibres. The nuclei of the myotubes remained in an internal position, and in only one myotube were cross-striations faintly visible (Fig. 4*b*). These myotubes bore a striking resemblance to those of uninnervated muscle in culture^{11,14}. One nerve ending was observed morphologically, but did not appear to terminate on a myotube, and there was no localization of acetylcholinesterase activity.

Quantitative assessment of the regeneration of the cultures after 3 weeks *in vitro* showed that there was a significant difference in the formation of myotubes and muscle fibres between the muscle coupled with normal and "dystrophic"

Table 1 Percentage of Cultures with Myotubes during First 3 Weeks *in vitro*

Spinal cord	Muscle	Total No. of cultures	% (No.) of cultures with myotubes	Mean % $\pm$ s.d. for group	Significance
Normal	Normal	10	90 (9)	93.5 ± 5.8	$P < 0.001$
Normal	Dystrophic A*	11	100 (11)		
Normal	Dystrophic B†	10	90 (9)		
Dystrophic B	Normal	6	17 (1)	23.0 ± 8.0	
Dystrophic B	Dystrophic A	4	25 (1)		
Dystrophic B	Dystrophic B	3	33 (1)		

* 129/reJ-day; † C57 Bl/6J-day^{2J}

cord (Table 1). Qualitative differences were also observed between these myotubes of the two groups; those from muscle coupled with "dystrophic" cord did not appear to be functionally innervated, whereas those from muscle innervated by normal cord showed many of the characteristics of mature muscle fibres. Contractile activity was also qualitatively different in the two groups.

These results show two things. First, that murine dystrophic muscle behaves morphologically quite normally when regenerating in the presence of normal spinal cord; and second, that regeneration of both normal and dystrophic muscle is severely affected when coupled with spinal cord from dystrophic mice. The state of the muscle is therefore determined by that of the spinal cord. The absence of any humoral, vascular, or higher central nervous influences in this experimental model would suggest that neurones in the spinal cord are by themselves capable of producing the myopathic state of the muscle. The neurones and neuritic growth of the "dystrophic" spinal cord appeared to be morphologically normal; yet functional neuromuscular junctions did not develop. A chemical or physiological fault may thus be responsible.

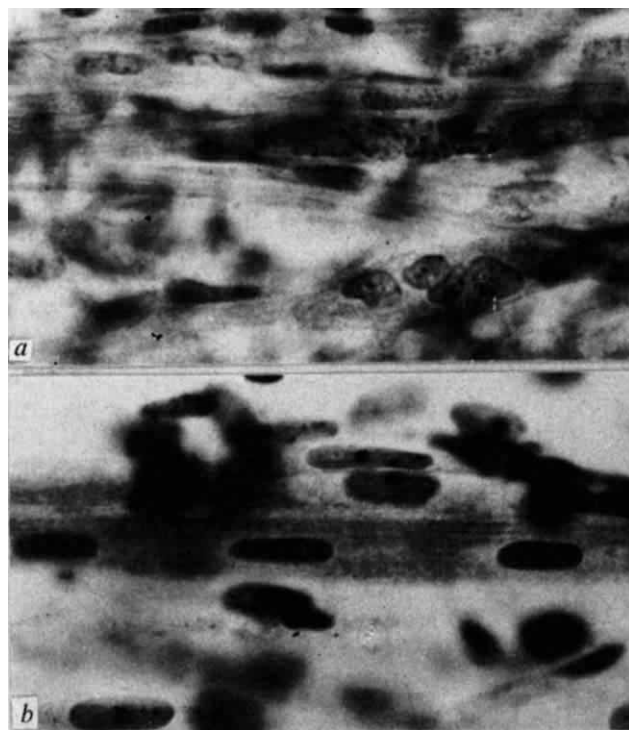


Fig. 4 Regenerated myotubes of muscle coupled with "dystrophic" spinal cord; 21 d *in vitro*. *a*, Normal muscle. Verhoeff-Van Gieson stain, $\times 800$. *b*, Dystrophic muscle. Holmes's silver impregnation, $\times 800$.

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Collagen Synthesis in Long-term Amniotic Fluid Cell Cultures

CYTOLOGICAL investigations indicate different possible origins of amniotic fluid cells (AFCs). The upper respiratory, gastrointestinal and genitourinary tracts, the skin, amnion and umbilical cord^{1,2} are all considered to be sources of AFC. The growth of epithelioid and fibroblastoid cells is common³ in the early stages of culture, and during further cultivation the cells are characterized by uniform fibroblastoid morphology, but their origin is not yet clear^{4,5}. Our preliminary results in two human AFC lines suggest evidence of collagen synthesis in these cultures and include the examination of ratios of soluble to insoluble collagen⁶.

We have studied collagen synthesis in five AFC cultures (Table 1). The AFCs were obtained by amniocenteses in the course of sectio minor and cultivated in EPL medium⁷ with 10% calf serum and Eagle's minimal essential medium (MEM) vitamins⁸. After the first passages the cells were

propagated in this medium without serum; the E26 cells were grown without serum from the start of cultivation. The male sex of cultivated E14 and E15 AFCs derived from male fetuses was proved by direct cytogenetic examination, by fluorescence determination of Y chromosome⁹ and the F body in 34 to 40% of cells, eliminating the possibility of contamination by maternal fibroblast in the cultures under investigation.

The cells for biochemical investigation were collected in the stationary phase, 7 to 10 d after subcultivation during 10 to 24 weeks of *in vitro* cultivation in particular lines by the methods described earlier¹⁰ (Table 1). Total collagen corresponds to the sum of hydroxyproline in isolated collagen fractions and collagen in the supernatant after centrifugation of cell suspensions obtained from AFC cultures. Soluble and insoluble collagen fractions were isolated from the cell pellet. Cell nitrogen is the sum of nitrogen in the supernatant after centrifugation of the original cell suspension, nitrogen in isolated collagen fractions and in the residue of cells after extraction with hot trichloroacetic acid (see Table 1).

During the phase of primoculture actively growing epithelioid colonies were seen, gradually exhibiting fibroepithelioid characteristics. After the first passages, they were characterized by typical uniform fibroblast morphology in monolayer cultures.

The fibroblast differentiation was proved by collagen synthesis in all lines under investigation (Table 1). The hydroxyproline/N ratio varied between 2.6 and 5.52, with the average value 3.83 except for the E8 culture, where the hydroxyproline/N ratio reached 9.13. This deviation may reflect the individual variability depending on the donor and culture derived. This possibility is suggested by finding a similarly high value for the hydroxyproline/N ratio in parallel E8 fibroblast cultures derived from foetal peripheral blood¹¹. Values higher than the average range were also found for some samples from the many investigations reported previously. The observed values of hydroxyproline/N correspond well with the variation range (3.3 ± 0.3) found in other types of long term cultivated human diploid fibroblast lines^{12,13}. The collagen synthesis of fibroblast cultures derived from AFC and amnion was nearly identical. The mutual proportions of soluble to insoluble collagen exhibit variability in particular lines in the range 0.44–1.43, which are in good agreement with variation range (1.23 ± 0.67) found in other types of long term cultivated human diploid cell lines¹⁰.

The demonstration of collagen synthesis in the AFC cultures investigated identifies the long term cultivated cells as fibroblasts. Similar characteristics of collagen synthesis and maturation as in human diploid fibroblastic cell lines are evidence for the mesenchymal origin of long term cultivated amniotic fluid cells; collagen synthesis by mammalian cell lines of non-fibroblastic origin is minimal¹⁴. These cultures

Table 1 Collagen Synthesis in Amniotic Fluid Cell Cultures

Foetus	Age (weeks)	Sex	Length of cultivation (weeks)	Total (μ g hydroxyproline/mg N)	Collagen		NSC/ISC
					Soluble (μ g hydroxyproline)	Insoluble (μ g hydroxyproline)	
E2	20	♀	10	3.76	—	—	—
E2 AMN				4.34	—	—	—
E8	16	♀	14	9.13	48.3	109.5	0.44
E14	24	♂	11	2.61	4.33	3.03	1.43
E15	16	♂	12	5.52	20.3	40.7	0.50
E26	24	♀	19	2.93	8.57	7.45	1.15

Nitrogen (N) was determined by nesslerization. Hydroxyproline was ascertained by the modified Prockop and Udenfriend's method¹⁵ after acid hydrolysis (6 N HCl, 105° C for 16 h). Soluble collagen (NSC) refers to collagen extractable at 4° C into 0.7 M NaCl, pH 7.2. Insoluble collagen (ISC) was extracted by hot trichloroacetic acid¹⁶, and corresponds to collagen remaining after the extraction with NaCl. AMN, parallel culture derived from the amnion.

could possibly be used, therefore, for the prenatal investigation of hereditary disorders of human connective tissue.

The amniocenteses were carried out in the Third Department of Gynaecology and Obstetrics, Faculty of Paediatrics, Charles University, Prague.

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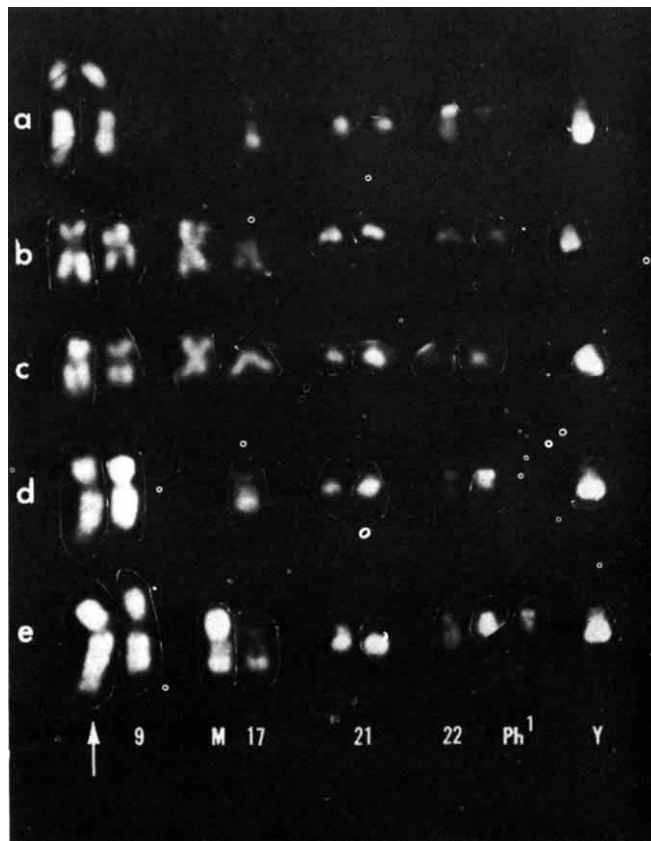


Fig. 1 Partial karyotype of five mitotic cells from cases 1-3 photographed with quinacrine fluorescence. The first pair of chromosomes in each row is the 9, with the 9q+ chromosome identified by the arrow. Note the extra band of dully fluorescing material at the end of each 9q+ chromosome. The next chromosome is the metacentric marker chromosome (M), when one is present, paired with the normal chromosome 17. The pair of chromosomes 21 is followed by the normal chromosome 22 and the Ph¹ chromosome. The Y chromosome is the last one in each row. *a*, Cell obtained from case 1. *b* and *c*, Cells obtained from case 2. The upper arm of the marker has two bands including one near the centromere, which would not be expected in an isochromosome 17. *d*, Cell obtained from case 3 before transformation. Note the relatively bright short arm region of the Ph¹. *e*, Cell obtained from case 3 after transformation. One arm of the marker appears to be homologous to the long arm of 17; the other arm shows relatively even moderate fluorescence. The two Ph¹ chromosomes are arranged with that similar to the initial Ph¹ being placed first.

A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia identified by Quinacrine Fluorescence and Giemsa Staining

CELLS from nine consecutive patients with chronic myelogenous leukaemia (CML) have been analysed with quinacrine fluorescence and various Giemsa staining techniques. The Philadelphia (Ph¹) chromosome in all nine patients represents a deletion of the long arm of chromosome 22 (22q-)^{1,2}. An unsuspected abnormality in all cells from the nine patients has been detected with these new staining techniques. It consists of the addition of dully fluorescing material to the end of the long arm of one chromosome 9 (9q+). In Giemsa-stained preparations, this material appears as an additional faint terminal band in one chromosome 9. The amount of additional material is approximately equal to the amount missing from the Ph¹ (22q-) chromosome, suggesting that there may be a hitherto undetected translocation between the long arm of 22 and the long arm of 9, producing the 9q+ chromosome.

Bone marrow specimens were prepared for chromosomal analysis as previously described³. We cultured peripheral blood leukocytes for 24 or 48 h without phytohaemagglutinin to

obtain circulating immature leukaemic cells in division, and for 72 h with phytohaemagglutinin to obtain lymphocytes in division. The time in Velban® was reduced to 40 min for bone marrow and 60 min for peripheral leukocytes so that extended chromosomes, which are more useful for quinacrine fluorescence and Giemsa staining, could be obtained.

Some slides were used for the initial analysis and then destained as previously reported⁴, while other slides were prepared from material which had been stored for several weeks to 9 months at -20° C in fixative (45% glacial acetic acid, 55% methanol). All slides, either newly prepared or destained, were stained in a solution of quinacrine mustard (approximately 50 µg/ml.) dissolved in McIlvaine's buffer at pH 5.5 for 30-60 min, rinsed twice in buffer, pH 5.5, mounted in buffer, and examined as previously described⁵. Well-spread metaphases were photographed with a 95X fluoride objective containing an iris diaphragm on Kodak Panatomic X film using exposure times of 8-15 s. After the coverslips had been removed and the slides dehydrated through 70% and 95% alcohol, they were stained with Giemsa (Gurr's Giemsa R66) directly or after treatment either with the ASG technique⁶ or with NaOH⁷. Six to fifty cells from each patient were analysed with both quinacrine

fluorescence and Giemsa stain. Duplicate karyotypes were prepared according to the Chicago¹ and Paris² nomenclature from photographs of quinacrine fluorescence and Giemsa-stained cells in metaphase.

The patients had had CML for 2 months to 6 years, and all had responded well to busulphan. Case 2 had received prednisone and 6-mercaptopurine one year before cytogenetic analysis. Cases 2, 4, and 5 were studied only in the acute blast phase, whereas cases 1 and 3 were studied in remission as well as in the acute blast phase of CML. Case 3 showed a change in modal chromosome number from 46 in remission to 50 in the terminal phase of his disease. Cases 6 and 7 were in the early phase, and cases 8 and 9 were in remission at the time of analysis. Cultures of peripheral lymphocytes from five of the patients, three of whom were in blast crisis, have provided no mitotic cells suitable for analysis. No cultures were taken from the remaining patients. Cytogenetic findings are summarized in Table 1.

Analysis of cells from all nine cases confirmed previous reports^{8,9} that the Ph¹ chromosome was a deleted 22 (Fig. 1). All cells examined with fluorescence showed that one chromosome 9 had additional dully fluorescing material at the terminal portion of the long arm (9q+) (Figs. 1, 3, and 5). Giemsa-stained cells showed an additional faint terminal band on the same chromosome 9 (Figs. 2 and 4). This change was seen in cells from case 3 obtained before blast crisis (Figs. 1*d* and 2*d*), as well as in samples obtained in the blast phase (Figs. 1*e*, 2*e*, and 3). The additional material is approximately equal to one-half the length of the moderately fluorescing region of the long arm of 9.

The occurrence of an apparently identical chromosomal abnormality in addition to the Ph¹ chromosome in nine patients with CML indicates that the chromosomal pattern in CML is not as simple as it has appeared to be. The source of the dully fluorescing and pale-staining material on the 9q+ chromosome cannot be determined at present. Its appearance is similar to that of the long arm of chromosome 22, which suggests that it may represent a translocation of the portion of chromosome 22 that is missing from the Ph¹ chromosome. In support of this suggestion, the amount of material deleted from 22 is approximately equal to the additional dully fluorescing portion observed on the 9q+ chromosome. Furthermore, other large regions of dull fluorescence, such as the end of the short arm of chromosome 1 or the end of the long arm of chromosomes 12 or 15, which could be the source of this material, appear to be intact.

The mechanism for the production of such a specific chromosomal translocation (if this is the correct explanation for these findings) is not clear. This would constitute the only specific chromosomal translocation in humans that has been

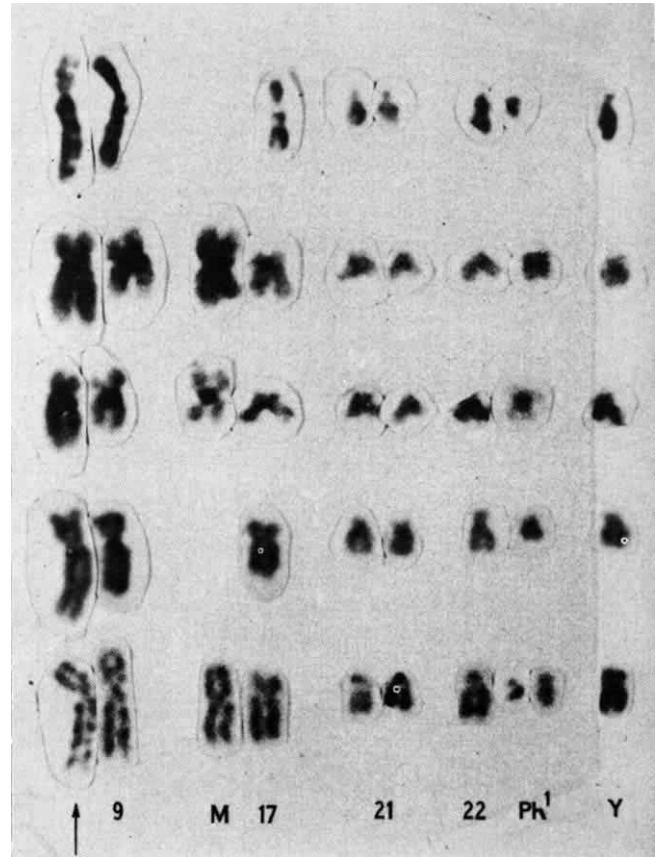


Fig. 2 Partial karyotype of the same five mitotic cells shown in Fig. 1, stained with Giemsa. The arrangement of the chromosomes is as in Fig. 1. Note the additional faint band of material at the end of each 9q+ chromosome (↑). *a*, Slide destained after quinacrine and restained with Giemsa (Gurr's R66) 1:10 for 10 min. There is a suggestion of banding although the preparation was not specially treated to produce bands. *b*, Slide destained after quinacrine, incubated in 2×SSC for 2.5 h, and stained with Giemsa. Note the fine band near the centromere region in the upper arm of the marker chromosome, which is not present in the lower arm. *c*, Slide destained after initial Giemsa stain and again after fluorescence; treated with 0.07 N NaOH for 1 min and incubated in 2×SSC for 2.5 h. Note the relatively large amount of centromeric heterochromatin in the marker, extending into the upper arm which may not be homologous to 17q. *d*, Slide destained after quinacrine and incubated in 2×SSC for 2.5 h before staining with Giemsa. *e*, Slide destained and incubated in 2×SSC for 2 h and stained with Giemsa. Note the similarity of bands in the lower arm of the marker with those in chromosome 17, and the longer long arm in the second Ph¹ chromosome as compared with the original.

Table 1 Summary of Chromosomal Analysis

Case	Age (yr)	Duration of CML (yr)	Source	Number of cells analysed with quinacrine and Giemsa	Karyotype ^{a,b,7}	Figure number
1§	72	6	Marrow	8	46,XY,9q+,22q-	1a, 2a
2§	29	3½	Marrow	25	48,XY,9q+,+C,+mar,-17,+?F,22q-	1b,c, 2b,c
3§	37	3½	Marrow†	15	46,XY,9q+,22q-	1d, 2d
			Blood	26	50,XY,9q+,+8,+C,+mar,22q-,+22q-	1e, 2e
			Marrow	10	50,XY,9q+,+8,+C,+mar,22q-,+22q-	5
4§	71	1½	Blood	6	46,XX,9q+,+mar,-17,22q-	3a, 4a
				3	47,XX,9q+,+C,+mar,-17,22q-	
5§†	51	2½	Blood	50	48,XY,9q+,+mar,22q-,+22q-	3b, 4b
6	45	2 mo	Marrow	6	46,XX,9q+,22q-	3c, 4c
7	25	1	Marrow	12	46,XX,9q+,22q-	3d, 4d
8	18	3	Marrow	21	46,XX,9q+,22q-	3e, 4e
9	64	3½	Marrow	11	46,XX,9q+,22q-	3f

* (+) additional; (-) absent; (q) long arm; (mar) marker; (22q-) represents the Ph¹ chromosome. † Obtained before blast crisis. ‡ Slides provided by Dr Lorraine F. Meisner, State Laboratory of Hygiene, Madison, Wisconsin. § Patient died in blast crisis.

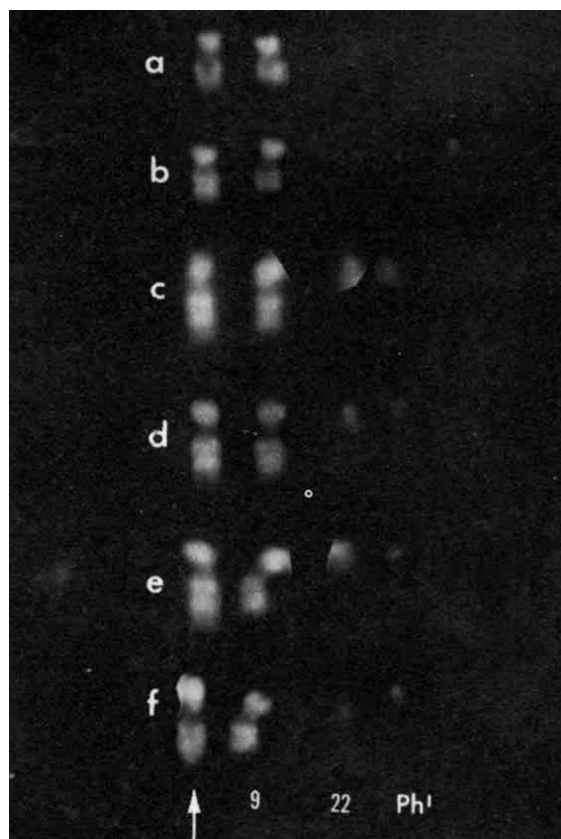


Fig. 3 Partial karyotype of six mitotic cells from cases 4-9 photographed with quinacrine fluorescence. The first pair of chromosomes in each row is the 9, with the 9q+ chromosome identified by the arrow. Note the extra band of dully fluorescing material at the end of each 9q+ chromosome. The second pair of chromosomes is the normal 22 and the Ph¹ chromosome. The cells were obtained from case 4 (a), case 5 with two Ph¹ chromosomes (b), case 6 (c), case 7 (d), case 8 (e), and case 9 (f).

identified. Previous attempts to determine whether the Ph¹ chromosome represented a loss of chromosomal material rather than a translocation floundered because it was not possible to identify precisely all the human chromosomes¹⁰. The 9q+ chromosome was observed in case 3 before the acute blast phase, as well as in cases 6-9 who were in remission. This suggests that the 9q+ chromosome may be present in the initial development of the somatic mutation, and that the translocation is not solely a result of progressive changes in the karyotype which frequently occur in later stages of blast crises.

Patients with CML having cells with the Ph¹ chromosome fare better clinically than patients who do not have this abnormality^{11,12}. If it is assumed that the 9q+ represents a translocation from 22, then leukaemic cells with or without the Ph¹ chromosome appear to have approximately the same amount of chromosomal material. In this case, differences in the clinical course cannot be accounted for by a gross loss of chromosomal material. The assumption that the additional material on chromosome 9 comes from 22 can be tested by examination of the chromosomes from patients with CML whose cells are Ph¹ negative.

For reasons that are not understood, chromosomes from leukaemic cells are often "fuzzy" with poorly defined centromere regions. This makes accurate karyotyping of cells from leukaemic patients difficult and sometimes impossible. Nevertheless, in the present study using quinacrine fluorescence and Giemsa stains, additional findings have emerged. For example, case 3 in the terminal phase showed two Ph¹ chromosomes, but these were not identical. One consistently had a brightly fluorescing short arm region similar to that of the Ph¹ chromo-

some found in the analysis before blast formation. The other Ph¹ chromosome showed dull fluorescence in the short arm region and had more material in the long arm. Case 5 also had two Ph¹ chromosomes, but they were identical in size and fluorescence pattern.

Two other findings are of interest: (1) The extra C group chromosomes in two patients in blast crisis (cases 2 and 3) most closely resemble chromosome 8 in size, centromere index, and general fluorescence pattern. (2) At least one arm of the metacentric marker chromosome found in cases 2, 3, and 4 appeared to be homologous to the long arm of 17 (17q), and the marker in case 4 may be an isochromosome for the long arm of 17 (17qi). Lobb *et al.*¹³ observed a metacentric marker chromosome in three patients who had CML and, with the use of the ASG technique, identified the marker as an isochromosome for the long arm of 17. The apparent frequency of metacentric marker chromosomes occurring at the time of acute blast crisis and involving the long arm of chromosome 17 remains an enigma.

As has been stated earlier⁷, a combination of quinacrine fluorescence and various Giemsa staining techniques provides much more information than any one technique used separately. Application of these new techniques to the study of other types of tumours has also been fruitful. Analysis of cells from a patient with acute myelogenous leukaemia not only permitted identification of the chromosomes involved in a translocation, but also pointed to the sites of chromosomal breakage¹⁴. An extra terminal bright band on chromosome 14 has been observed recently by Manolov and Manolova¹⁵ in biopsies and cell cultures from Burkitt's lymphomas in ten out of twelve

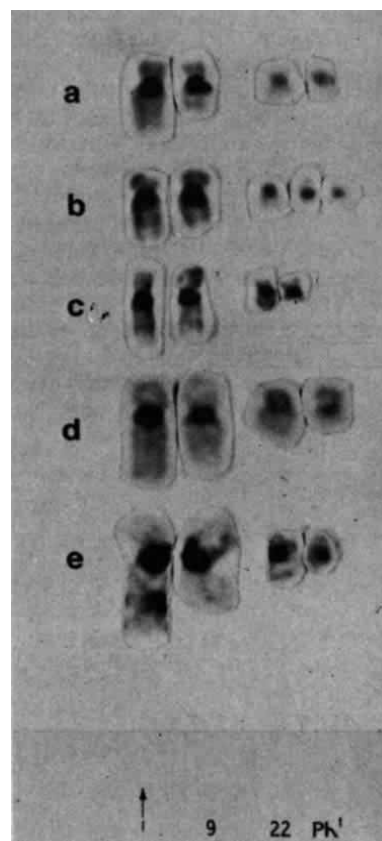


Fig. 4 Partial karyotype of five of the six mitotic cells shown in Fig. 3, stained with Giemsa. All slides were treated with 0.07 N NaOH for 2 min, incubated in 2×SSC at 60° C overnight, and stained with Gurr's Giemsa R66 1: 20 for 30 min. Note the additional faint band of material at the end of each 9q+ chromosome, and the 2 Ph¹ chromosomes in case 5 (b). Giemsa preparations for case 9 were unsuccessful.

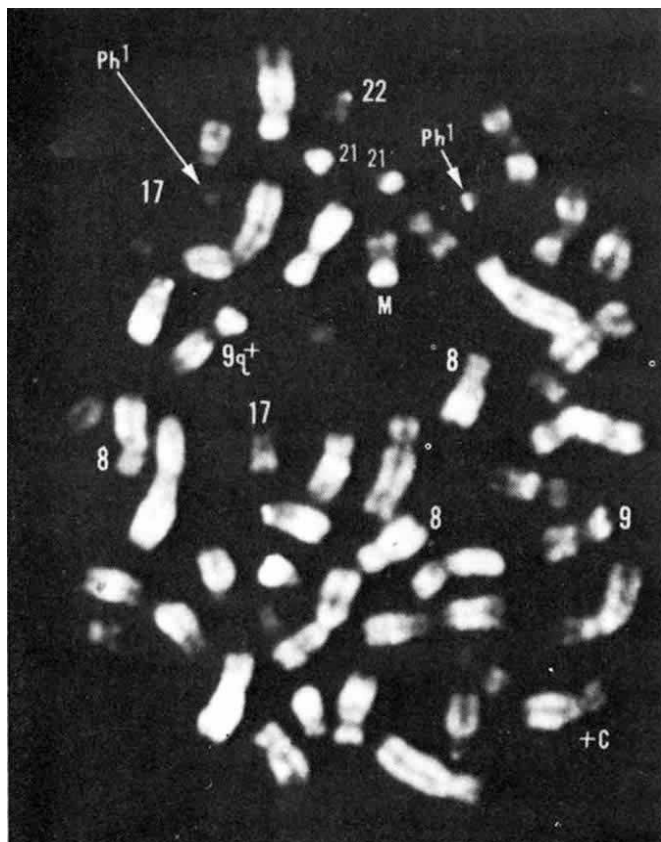


Fig. 5 Intact metaphase from case 3 stained with quinacrine mustard. This cell contains 50 chromosomes, including two extra C chromosomes, the metacentric marker, and two Ph¹ chromosomes. The 9 and 9q+ chromosomes are identified. One of the two extra C chromosomes is identical to an 8, and therefore three chromosome 8s are identified; the relative similarity in size and fluorescence pattern between the other C chromosome (labelled +C) and chromosome 8 can be observed. The arm of the metacentric marker (M) away from the label is 17q. The normal 17s are labelled, as are the normal 21s and the 22. The original Ph¹ is identified by the short arrow, and the second Ph¹ by the long arrow. In each case, the arrow points to the short arm of the chromosome. The brighter short arm and shorter long arm of the original Ph¹ chromosome as compared with the second Ph¹ are clearly demonstrated. (The difference in brightness between the two Ph¹ chromosomes seen here was noted consistently in all mitotic cells, whereas the dullness of the peripheral chromosome 17 was an artefact of this cell.)

patients. The loss of chromosome 22 in meningiomas has been confirmed with fluorescence techniques¹⁶.

Whereas it appeared earlier from the bizarre chromosomal abnormalities identified in leukaemic cells that almost any kind of genetic imbalance might be viable, the new techniques may well demonstrate that excesses or deficiencies of only certain chromosomes can be tolerated. Careful analysis of cells from patients with leukaemia may identify certain chromosomal regions that are frequently aneuploid in leukaemic cells. This information could contribute to an understanding of the role of chromosomal changes in malignancy.

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Experimental Model for von Willebrand's Disease

It has been reported¹ that Ristocetin induces aggregation of platelets in normal platelet-rich plasma (PRP) but not of platelets from patients with von Willebrand's disease. Results presented here demonstrate that the aggregation of platelets induced by Ristocetin involves a factor present in normal and haemophilia A plasma and absent in Willebrand plasma. Lack of such a factor would then explain the inability of platelets to aggregate in response to Ristocetin in von Willebrand's disease. In eight patients fulfilling the criteria of von Willebrand's disease (Table 1), platelet aggregation induced by Ristocetin was markedly deficient when compared to healthy normal plasmas or did not occur at all. The abnormal responses of platelets from von Willebrand's patients could be corrected by the addition of small volumes of normal platelet-poor plasma (PPP) (Fig. 1); similar correction was achieved by Haemophilia A PPP but not by PPP from patients with von Willebrand's disease.

Table 1 Biological Data in Eight Patients with von Willebrand's Disease

Patients	Platelet retention %	Factor VIII activity %	Factor VIII-like antigen %	Initial velocity of aggregation to Ristocetin	
				2 mg ml ⁻¹	1 mg ml ⁻¹
1	15	37	38	4.5	0
2	6	4	<5	0	0
3	8	10	<5	0	0
4	0	3.8	<5	0	0
5	27	10	<5	0	0
6	12.5	51.5	8	30*	3
7	0	20	<5	26.5*	0
8	0	25	<5	51*	9*
Controls	80-100	50-200	50-200	70-118	24-67

* Lag phase of 15 s.

Platelet retention to glass beads was measured by the method of Bowie *et al.*² and Factor VIII antigen was assayed on plasma by a modification³ of the Laurell technique⁴. Platelet aggregation was studied in the MK III aggregometer⁵ by the turbidimetric method⁶ using citrated PRP. The initial velocity of aggregation was obtained by drawing a tangent to the steepest part of the aggregation curve on a potentiometric chart recorder and expressed as a percentage of the maximum transmitted light (PPP) after 30 s.

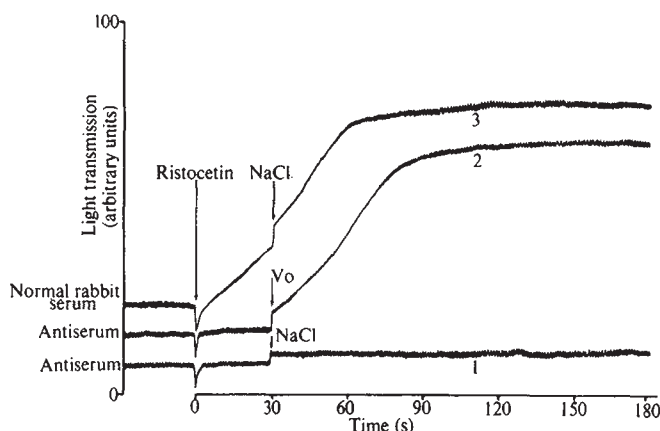


Fig. 1 Effect of normal platelet-poor plasma on Ristocetin-induced platelet aggregation in von Willebrand's disease. 0.8 ml PRP was incubated for 3 min at 37° C, with stirring, with: 1, 0.2 ml 0.154 M sodium chloride; 2, 0.05 ml normal PPP + 0.15 ml 0.154 M sodium chloride; 3, 0.10 ml normal PPP + 0.10 ml 0.154 M sodium chloride; or 4, 0.20 ml normal PPP; 5, 0.8 ml normal PRP + 0.2 ml 0.154 M sodium chloride. 10 μ l of Ristocetin (2 mg ml⁻¹ final concentration) was then added.

Here we report the development of an experimental model for von Willebrand's disease, based on the inhibitory effect of a specific heterologous antiserum on aggregation induced in normal PRP by Ristocetin. The antiserum was raised in rabbits using as antigen material concentrated from the void volume (V_0) fraction of High Purity AHF concentrate⁷ after gel filtration through 'Sephacrose' 4B. The fraction contained both Factor VIII activity (60 U mg⁻¹) and Willebrand Factor, because it corrected abnormal platelet retention to glass beads in patients with von Willebrand's disease (ref. 8 and unpublished work by D. M.). This antiserum neutralizes Factor VIII activity of normal plasma (Titre 80 Oxford U ml⁻¹); is precipitating and specific; reveals by immunodiffusion and two-dimensional electrophoresis⁹ the presence of one single component in normal and Haemophilia A plasma but not in von Willebrand plasma³; and decreases platelet retention of normal blood to glass beads⁸.

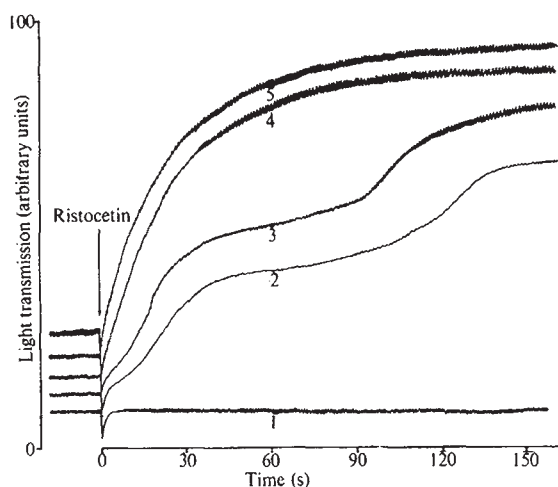


Fig. 2 Correction by the void volume (V_0) fraction of the inhibitory effect of anti-Willebrand and/or Factor VIII-like antiserum upon Ristocetin-induced platelet aggregation. Samples were incubated for 3 min at 37° C, with stirring before the addition of 10 μ l of Ristocetin (1 mg ml⁻¹ final concentration), followed after 30 s by 30 μ l of either V_0 or 0.154 M sodium chloride. 1, 1 ml PRP + 30 μ l antiserum + 10 μ l Ristocetin + 30 μ l sodium chloride; 2, 1 ml PRP + 30 μ l antiserum + 10 μ l Ristocetin + 30 μ l V_0 fraction; 3, 1 ml PRP + 30 μ l normal rabbit serum + 10 μ l Ristocetin + 30 μ l sodium chloride.

The specific heterologous antiserum inhibits platelet aggregation in normal PRP caused by Ristocetin (Table 2). The degree of inhibition depends on the volumes of the antiserum added: total inhibition of platelet aggregation was achieved when 30 μ l of antiserum was added to 1 ml PRP. Control experiments were performed with the use of same volumes of heterologous serum from the same non-immunized rabbit (Table 2), anti-human fibrinogen antiserum or 0.154 M sodium chloride. In no case was platelet aggregation induced by Ristocetin affected. The inhibitory effect of the antiserum was overcome by the addition of normal and Haemophilia A plasma and by the same highly purified fraction eluted in the void volume which was used to raise the antiserum (Fig. 2), but not by cryoprecipitate prepared from von Willebrand plasma or from normal or Haemophilia A plasma heated for 30 min at 56° C.

Table 2 Platelet Aggregation to Ristocetin (1 mg ml⁻¹ Final Concentration) of Normal Human PRP. Effect of Anti-Willebrand Factor and/or Factor VIII-like Antiserum (Mean of Five Experiments)

Volume added (μ l)	Initial velocity % Control (normal rabbit serum)	Rabbit antiserum
10	41	29
20	62	32
30	56	0

Human PRP (1 ml) was incubated with normal rabbit serum or specific antiserum for 3 min at 37° C, with stirring, before addition of 10 μ l of Ristocetin.

We do not know the mechanism by which Ristocetin initiates platelet aggregation. But it is different from aggregation induced by adenosine diphosphate (ADP) because aggregation by Ristocetin, unlike that by ADP, occurs in the presence of ethylenediamine tetraacetate (EDTA 0.15%)⁴, is not inhibited by adenosine (5×10^{-4} M) or prostaglandin E₁ (5×10^{-4} M) but is inhibited by the specific heterologous antiserum. These observations suggest that Ristocetin-induced platelet aggregation involves a plasmatic factor known as Willebrand Factor^{2,10} and/or Factor VIII-like antigen^{3,8,11-13}. Thus such an experimental model can be used for further characterization of Willebrand Factor and for the study of its identity and relationship to the Factor VIII-like antigen.

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Residual Visual Function after Brain Wounds involving the Central Visual Pathways in Man

It is generally believed that total destruction of visual cortex and optic radiations in man should lead to blindness, and that regional (subtotal) destruction should correspondingly produce circumscribed areas of blindness ("scotomata") in the visual field¹. Such areas of blindness are defined by a patient's inability to detect and report visual stimuli projected into the affected region. Standard methods of visual field testing (perimetry) suggest that such scotomata may be "absolute", that is the patient seems to be unable to distinguish between the presence and absence of visually presented targets whenever they are presented in the scotomatous area. Suspecting that the response of the visual system may depend on the task requirements, we used a technique which requires a localizing response from the patient², in addition to clinical perimetry. As a result, we have found evidence for the processing of information about the locus of light stimuli presented in areas of the visual field which are "blind" by the traditional definition.

Residual function in patients blinded by injury of the occipital lobes has already been established for the control of pupil size, which is known to be mediated by a reflex pathway through the pretectum³. Some older reports suggest that the blink reflex may still be intact in patients with complete "cortical" blindness^{4,5}. Brindley *et al.*⁶ reported that patients with total blindness of cortical origin can discriminate sudden changes in room illumination; ter Braak *et al.*⁷ were able to elicit optokinetic nystagmus in one such patient; and Richards⁸ suggested that visual information presented in a "cortically" blind area is still accessible to provide a primitive form of stereopsis. In subhuman species after lesions of the visual cortex, various residual visual capacities can be demonstrated which, however, may differ from species to species⁹⁻¹⁴.

This report is based on experiments performed with four patients who had scotomata within their visual fields owing to traumatic (three patients) or vascular (one patient) lesions within the occipital region of their brains. Using the Tübinger perimeter¹⁵, the visual field of each eye was carefully mapped to determine accurately the area in which the patient did not report seeing any visual target. One patient had no light perception in the right upper quadrants (A. W.), one in the left upper quadrants (L. H.), one in the right lower quadrants (W. D.), and one in the left lower quadrants with considerable involvement of the left upper quadrants (R. O.); thus, none of the patients had a complete hemianopia.

A meridian was chosen within the blind area along which visual targets were presented at various angular distances from the centre of gaze. The task of the patient was to localize by a non-verbal response the position at which the visual target had appeared; he was told to move his eyes to the position where he "guessed" the light had been presented. Patient A. W. was tested along the meridian of 30°, L. H. along 150°, R. O. along 180°, and W. D. along 330°; all patients were tested monocularly and the targets were always presented in the temporal visual fields.

In the experiment, the patient fixated in primary position a light spot subtending 10 arc min visual angle at a distance of 33 cm. This fixation point had a luminance of 100 mlambert and was projected on a homogeneous background of 1 mlambert luminance (low photopic range). The visual target whose position had to be identified was a circular white patch subtending 116 arc min visual angle and of 10 mlambert luminance; the lights were produced by tungsten bulbs. The target was flashed for 100 ms using a shutter; during this time the target moved from its initial position to a point approximately 4° further away from the fixation point. The angular separation of the target positions was

either 5° (W. D., R. O.) or 10° (L. H., A. W.); no targets were presented closer than 10° from the fixation point. The sequence of target positions was random. All four patients had intact foveal vision. They had no problems in holding fixation during the experiment. Fixation could be monitored in two ways: by direct observation through a small hole in the perimeter and by the electro-oculogram which was used to record the eye movements of the patients.

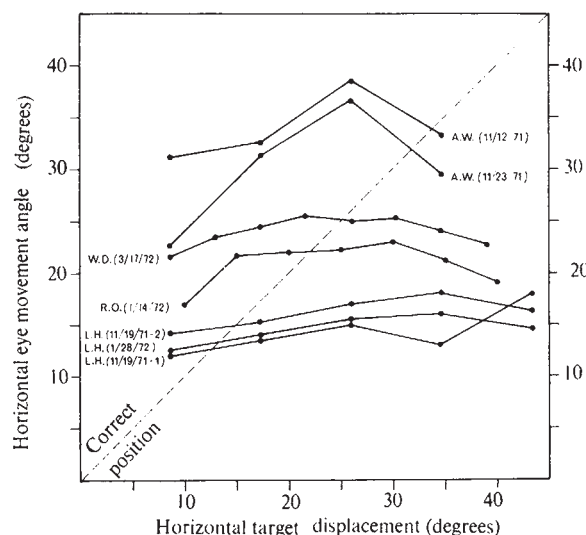


Fig. 1 Amplitude of saccadic eye movements as a function of the eccentricity of visual stimuli in patients with visual field defects owing to lesions of the occipital lobes. Targets were presented along the 30° meridian (A. W.), 150° meridian (L. H.), 180° meridian (R. O.), and 330° meridian (W. D.). R. O. and W. D. were tested once, A. W. and L. H. were tested on two different days, L. H. was tested twice on one day. The data are based only on the horizontal deviations of the eyes; a 10° eye movement along any of the oblique meridians has a horizontal component of 8.66° (10 times the cosine of 30°). Each point is the arithmetic mean of four to seven measurements. For each patient the average data points at different eccentricities are connected with each other. Note the increase of average eye movement amplitude for targets presented between 10° and approximately 30° eccentricity.

As the patients had no light perception within their scotomata, they were not able to "see" the visual stimuli to which they had to respond. Therefore, a secondary stimulus, a click from a sound source at constant position, was sounded simultaneously in order to elicit the patient's response. The click signalled to the patient that a light was being presented within his blind area and that he should move his eyes along the specified meridian to what he "guessed" was the position of the flash. He was told to move his eyes immediately, hold the deviation of his eyes for approximately 1 s, and then return his eyes to the fixation point. (For some patients this task was puzzling; one commented: "How can I look at something that I haven't seen?") As the latency of saccadic eye movements¹⁶ that would bring the target into the seeing field of vision is longer than the presentation time of the target (approximately 200 ms latency for visually elicited saccades compared with 100 ms for the target presentation time), the patients never reported seeing any targets during the experimental procedure.

The eye movements were recorded using standard d.c. electro-oculography. Because we were unable to obtain high quality records of the vertical components of eye movements, the data presented here are based only on the horizontal components. Calibrations were obtained at the end of an experiment by instructing the patient to look back and forth

between the fixation point and continuously lit targets at different eccentricities along the horizontal meridian; these measurements were then used to determine the horizontal components of eye movements during an experiment. As all the oblique meridians used had an angle of 30° with the horizontal meridian, a target presented at 10° along any oblique meridian had a horizontal displacement of 8.66° , a target at 15° had a horizontal displacement of 12.99° and so on. The head was held on a chin rest during experimentation and calibration; a second observer verified that the patient did not move his head during either procedure.

Figure 1 summarizes the data of the experiments with the four patients. The horizontal component of the saccadic eye movements is plotted as a function of the target displacement along the horizontal meridian (abscissa). Each point corresponds to the arithmetic mean of the eye movement amplitudes for targets at any given position; the means are based on four to seven single measurements. Two patients (L. H. and A. W.) were tested on two different days, and one (L. H.) was tested twice on one day.

Clearly the patients could not identify the correct position of the targets. Each patient seemed to prefer a specific magnitude of saccadic eye movement which is similar for all target eccentricities. As is clear from the measurements in L. H. and A. W., the angle of preferred saccadic eye movements remains stable over several days or weeks.

For a given subject, if we compare the average eye position for targets closer to and farther away from the fixation point, it is evident that there is a small increase in average eye movement amplitude up to approximately 30° eccentricity along the horizontal meridian. In all seven curves in Fig. 1 the target presented at an angle of 10° from the fixation point (that is 8.66° horizontal target displacement in three patients) elicits the smallest displacement of the eye. Targets that are increasingly farther away from the fixation point lead on the average to increasingly larger eye movements; this increase is, however, limited to a central area of the visual fields beyond which eye movement magnitude (one exception) decreases again.

To evaluate the probability that the increase in average eye deviation with increasing eccentricity in the more central area of the visual field might occur by chance, some combinatorial considerations may be appropriate. For reasons of simplicity, only those eye movement amplitudes in each curve shall be considered which correspond to the three target positions closest to the fixation point. The ordering of three arithmetic means in one curve (the second point being greater than the first, the third greater than the second) has a chance probability of $1/(3!)$, that is $1/6$; as we observed the same ordering in four patients, the chance probability of our results is $1/(3!)^4$, that is $P < 0.001$; and if we consider all seven curves, assuming that the curves of the same patients (L. H. and A. W.) are statistically independent, the chance probability would be $1/(3!)^7$, that is $P < 10^{-5}$. The initial increase of eye movement amplitudes for targets with greater eccentricity is thus highly significant according to statistical convention.

In addition to these combinatorial considerations, a χ^2 analysis of the same data was performed. An analysis of variance could not be performed because of the inhomogeneity of the data (different meridians tested; different range of eye movement amplitudes for each patient). For each of the seven experiments the arithmetic mean of all single measurements for the three target positions closest to the fovea was determined. Then we counted the eye movements with an amplitude above or below the arithmetic mean, separated according to the three target positions. The values of the seven experiments were then added and analysed using the χ^2 technique. Under the null hypothesis (no dependence of the eye movement amplitude from the eccentricity of the targets) one would expect an equal number of eye movements above and below the arithmetic means

for the three target positions. The χ^2 analysis, however, rejects the null hypotheses ($\chi^2 = 9.83$; $df = 2$; $P < 0.01$) and suggests a significant result which means that there is indeed a systematic increase of the amplitude of eye movements with an increasing eccentricity of the targets for the three target positions closest to the fovea.

In control experiments we tested whether the observed effect was due to intensity discrimination of scattered light. Two patients with retinal scotomata tested in the same experimental conditions as the patients with cortical scotomata showed no increase in eye movement amplitude for more eccentric targets. Targets with different intensities (1, 10 or 100 mlambert) presented at a fixed position (10° eccentricity) to two patients with cortical scotomata did not lead to eye movements whose magnitudes were correlated with the intensity of the flashes. Thus, stray light is probably not responsible for the observed effect. When the auditory stimuli were not accompanied by visual stimuli ("catch" trials), the magnitude of saccadic eye movements was unrelated to the sequence of target positions used in the actual experiments. It is interesting to note that the patients had no feeling about the presence or absence of light stimuli; actually they believed that their performance was always completely random.

We propose that our observation of an increase in the amplitude of saccadic eye movements for more eccentric targets presented within the "cortically" blind areas of the patients' visual fields is mediated by some still functional central visual mechanism. Perhaps this effect is due to the midbrain in these patients; the midbrain is known to receive a substantial projection from the retina. Alternatively, there may remain in the visual cortex a representation of the visual field which is not revealed by ordinary perimetry but which is still accessible to a non-verbal response, that is orienting. If so, then our evidence would indicate an unsuspected form of dissociation of function.

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Lithium Secretion by the Salt Gland of the Goose

THE mechanism by which the salt gland of marine birds secretes a hypertonic solution of sodium chloride is of considerable interest. It is generally considered that the concentration gradient for sodium is established across the luminal membrane of the secretory cell by an electrogenic sodium pump¹, but Hokin², on the basis of measurements of the intracellular composition of salt gland slices *in vitro*, argued that the concentration gradient is established across the basal membrane. One of us (M. P.)³ repeated these experiments, however, and was unable to confirm Hokin's figures for the sodium and chloride content of salt gland cells. M. P. again proposed that sodium is actively transported from the intracellular fluid of low sodium concentration to the lumen of the tubules. Nevertheless, this does not preclude the possibility suggested by Hokin² that sodium and chloride pass into the cell across the basal membrane by an active mechanism involving exchanges for H^+ and HCO_3^- ; indeed, current evidence favours this view⁴.

Although Li^+ will stimulate membrane "transport" ATPase and active Na^+ sodium extrusion in some tissues⁴⁻⁶, it is itself not transported by the sodium pump in the same direction as Na^+ (refs. 5, 6). In other tissues, for example, frog skin⁷, toad bladder⁸ and frog muscle⁹, there are reports that Li^+ can be pumped, to some extent, in the same direction as active Na^+ movements. The movement of lithium in the avian salt gland, which can transport sodium at a very high rate, has therefore been investigated in order to determine (i) whether this ion is secreted and concentrated by the gland, (ii) where the electrochemical gradient is established, and (iii) if this site is similar to that for sodium. In view of the current interest in lithium as an agent of use in the treatment of psychological disorders, the results may be of wider interest.

In most studies experiments have been done *in vitro* and lithium has been added largely to replace sodium in the external medium. By infusing relatively low concentrations of lithium into the blood stream the normal external ionic environment of the cells is maintained and it seems that, at least for the periods used in the experiments described here, movements of lithium can be studied alongside those of other substances.

The methods used for studying salt gland secretion in conscious domestic geese (*Anser anser*) have been previously described¹⁰⁻¹². The four birds used in our experiments had catheters inserted into a brachial artery and a brachial vein. Salt gland secretion was induced by the intravenous injection of 0.5 M NaCl (18 ml kg^{-1} body weight). When secretion was established 0.5 M LiCl was infused intravenously at a rate of 1 ml min^{-1} ; blood samples were taken from the arterial catheter. The LiCl infusions were discontinued after 25 min because in earlier experiments it was found that if LiCl was infused for longer the birds became lethargic and eventually vomited. Nevertheless this period of time was sufficient for plateau concentrations of Li in arterial plasma to be reached.

In two experiments ¹⁴C-sucrose in 0.154 M NaCl was infused intravenously at 1 ml min^{-1} starting 10 min before the infusion

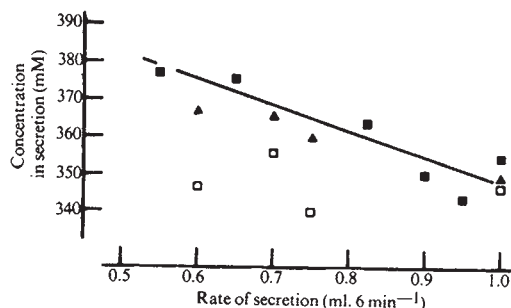


Fig. 1 Relationship between $[Na^+]$, $[Na^+ + Li^+]$ and rate of secretion in a goose before and during the infusion of 0.5 M LiCl (1 ml min^{-1}). Salt gland secretion was induced by the injection of 0.5 M NaCl (18 ml kg^{-1}) intravenously. The regression line shown was calculated from the points obtained before the infusion of LiCl. (■), $[Na^+]$ before infusion; (□), $[Na^+]$ during LiCl infusion; (△), $[Na^+ + Li^+]$ during LiCl infusion. Note that it was not possible to calculate a line for $[Na^+]$ or $[Na^+ + Li^+]$ when LiCl was being infused since plasma $[Li^+]$ was changing as a result of the infusion, and only the point at 0.6 ml per 6 min was obtained when equilibrium concentrations in plasma had been reached.

of LiCl, in order to determine the extracellular space of the salt glands. These birds were killed by injecting 600 mg of pentobarbitone sodium intravenously during the final minute of the LiCl infusion; the salt glands were then quickly removed for analysis. The analytical methods have been described previously^{3,11}. Li in plasma, nasal fluid and tissue digests was determined by atomic absorption spectrophotometry; allowance was made for interference by sodium.

In all four experiments the concentration of Li^+ in the secretion was higher than that in the plasma when equilibrium has been reached, the secretion/plasma ratio varying from 1.29 to 2.26 (Table 1). In the two experiments in which ¹⁴C-sucrose was also infused in order to enable measurement of extracellular space, the concentration of Li^+ in the non-extracellular tissue space, that is, intracellular and lumen (transcellular) spaces, was calculated to be 13.3 and 6.6 mmol l^{-1} water. As the concentrations of Li^+ in the secretion were higher than these figures (15.8 and 15.3 mM), this procedure slightly overestimates the true intracellular concentration.

The concentration of Li^+ in the secretion was higher than that of the intracellular fluid, and the lumen of the ducts of the gland is electrically positive with respect both to the inside of the secretory cells and to blood¹³. So it must be concluded that Li^+ , like Na^+ , is actively transported against both electrical and chemical gradients across the luminal cell membrane. There is certainly good physiological evidence to suggest the presence of a Na^+ pump at this site¹ and it seems likely that Li^+ is accepted and transported by this pump. This view is strengthened by the effects on the sodium concentration of the nasal fluid when lithium is being transported. In any one bird there is an inverse relationship between the Na concentration of the secretion and the rate of secretion¹¹. Fig. 1 shows that when Li^+ was being infused and transported, the figures for $[Na^+]$ fall below the line relating rate to concentration. When the values for $[Na^+]$ and $[Li^+]$ are added together, the

Table 1 Lithium Concentration in Plasma, Nasal Fluid and Intracellular Fluid (ICF) of Goose Salt Glands during the Infusion of 0.5 M LiCl Intravenously (1 ml min^{-1})

Goose	Plasma		ICF (mmol l^{-1} water)		Secretion		Secretion/plasma ratio		Secretion/ICF ratio	
	$[Na^+]$	$[Li^+]$	$[Na^+]$	$[Li^+]$	$[Na^+]$	$[Li^+]$	$[Na^+]$	$[Li^+]$	$[Na^+]$	$[Li^+]$
1	157	7.0	35	13.3	410	15.8	2.51	2.26	11.7	1.19
2	166	8.4	20	6.6	484	15.3	2.92	1.82	24.2	2.32
3	154	8.9	—	—	374	16.3	2.43	1.83	—	—
4	164	15.6	—	—	347	20.0	2.12	1.29	—	—

The figures given are those obtained for a 6 min period when a plateau concentration of Li^+ in plasma was reached. The secretion/ICF figures have been calculated on the assumption that both Na^+ and Li^+ are freely distributed in the ICF.

points fall much closer to the line obtained before the infusion of LiCl was begun. This implies that Li^+ and Na^+ are transported by the same mechanism. The affinity of the luminal pump, however, at the concentrations of lithium used in these experiments, seems to be very much greater for Na^+ than Li^+ .

As far as transport into the cell across the basal cell membrane is concerned, the movements of Li^+ give no clue to the mechanisms involved. In the absence of active Li^+ transport out of the cell, $[\text{Li}^+]$ would be expected to reach higher concentrations in the cell than in plasma because of the potential difference, and it is not possible to distinguish between passive Li^+ entry and possible Li^+-H^+ exchange¹.

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Malarial Parasites in Mammalian Platelets

It is generally accepted that mammalian plasmodia undergo their schizogonic cycle in red cells and hepatocytes. With the exception of macrophages, which often engulf parasitized red cells, and granulocytes, which rarely phagocytize parasitic debris, other cells of blood and bone marrow do not carry plasmodia.

Our previous studies in man showed a significant and consistent increase in the mean size of blood platelets during acute infection with *Plasmodium vivax* or *P. falciparum*¹. Those studies led to a prospective, systematic analysis of the morphology and function of platelets (thrombocytes) during and after spontaneous human malarial infections. A similar study was performed experimentally using the NK65 strain of *Plasmodium berghei* inoculated intraperitoneally into young albino ICR mice.

Samples of infected blood were obtained without anticoagulant (from venous blood in man and from cardiac blood in mice) and fixed immediately in buffered, 1.5% glutaraldehyde solution prewarmed at 37° C. After fixation had occurred, platelets were separated and concentrated by differential, two stage centrifugation. A pellet of platelets was prepared using plasma poor cells as in a matrix. After postfixation in osmium tetroxide the material was embedded in epoxy and ultrasections were further stained by uranyl acetate and lead citrate.

Apart from functional abnormalities the human thrombocytes showed multiple ultrastructural changes before anti-malarial therapy was instituted. In several of these thrombocytes there were large membrane-bound inclusions of granular or lamellar osmiophilic material, sometimes resembling myeloid

bodies. These inclusions were suggestive of degraded parasitic material, but I was unable to identify obvious parasites in the platelets of more than eighteen patients examined by electron microscopy.

The thrombocytes of the infected mice also had inclusions of the type seen in humans. I also found several obvious parasites within mouse platelets. Some parasites could be recognized as merozoites (Fig. 1) or trophozoites; schizonts were not found. As judged by morphology alone, some of these intrathrombocytic parasites appeared to be viable. Others showed different stages of degeneration, which often resembled the inclusions described above in mouse and human platelets. The parasitized platelets were only moderately enlarged. The organelles, such as granules, mitochondria, canaliculi and microtubules, were generally well preserved and could be easily recognized, although not necessarily in all sections.

To my knowledge plasmodia, or any other protozoa for that matter, have not previously been found in mammalian platelets. The nucleated thrombocytes of some non-mammalian vertebrates may be invaded by plasmodia: Zuckerman observed by light microscopy thrombocytes, heterophils, macrophages and lymphocytes invaded by *Plasmodium gallinaceum* in one chick embryo infected in experimental conditions². Intrathrombocytic parasites have also been described in saurian malaria by Thompson and Huff³ and by Telford⁴. Scorza has observed, by light and electron microscopy, sexual and asexual stages of *Plasmodium tropiduri* in the thrombocytes of some naturally or experimentally infected iguanas (*Tropidurus torquatus*)⁵; his description suggests that a complete schizogonic cycle of *Plasmodium* may occur in those nucleated "platelets".

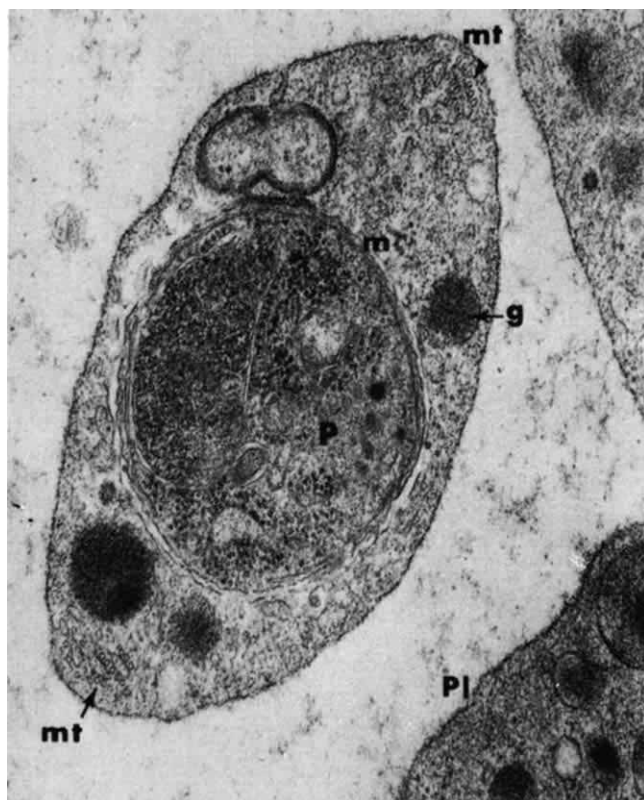


Fig. 1 Perpendicular section of a mouse platelet 2 weeks after intraperitoneal inoculation with *Plasmodium berghei*. Two granules (g) are present in the periphery of this thrombocyte. Circular profiles of the characteristic ring of microtubules (mt) are seen at the equator of the platelet. A plasmodium merozoite (P) is present in the centre, sharply separated from the platelet cytoplasm by concentric membranes (m). The nuclear mass (left) occupies a large portion of the parasite. Dense free ribosomes are scattered in the cytoplasm. A vesicular structure, lined by a double membrane, is near the centre. The cells partially shown in the right corners (Pl) are also platelets. Uranyl acetate and lead citrate, $\times 40,250$.

Parasites may reach the inside of the platelets either by active invasion—such as occurs when the conoid region of the merozoite penetrates the red cell—or through phagocytosis by the platelet. There is evidence that platelets are capable of engulfing such materials as thorotrast granules⁶, india ink⁷, starch⁸, and so on; masses of the size of these parasites have not, however, been seen in mammalian thrombocytes. As the platelets and parasites are fixed immediately after the blood is obtained, this finding cannot be the result of an *in vitro* phenomenon. It is unlikely that the parasites penetrate the cytoplasm of megakaryocytes and are then released within the individual platelet segments; I have examined a large number of sections of megakaryocytes in infected mice, and a few in humans, without finding any suggestion of parasites or inclusions.

The marked platelet enlargement proved in humans¹, and often seen in mice, is probably not the direct result of platelet invasion by parasites. The larger platelets, although abnormal by electron microscopy, do not show evidence of parasitic invasion. More likely the parasitic invasion of platelets could contribute to platelet depletion, which in malaria infections seems to be caused chiefly by disseminated intravascular coagulation^{9,10}. In turn this platelet depletion stimulates thrombopoiesis in the bone marrow with the release of large “young” thrombocytes^{1,11}.

This finding is too recent to speculate on its significance. By itself it indicates only that in this experimental mouse infection plasmodia can be found in platelets. By analogy it suggests that the same might occur in man.

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Head Movement of Flies during Visually Guided Flight

Most visually advanced animals are able to move their eyes relative to the body. This makes vision independent of body movements; in man it enables the eyes to keep the visual world stationary on the retinae, to scan the field of view, and to maintain fixation on moving objects¹. The extent to which animals other than vertebrates are able to perform these activities is largely unknown; many, especially arthropods, make body movements tending either to stabilize the visual surroundings^{2,3} or to cause fixation of objects by the eyes^{4,5}. In a very few cases, however, it has been shown that arthropod eyes may make directed movements independent of the body:

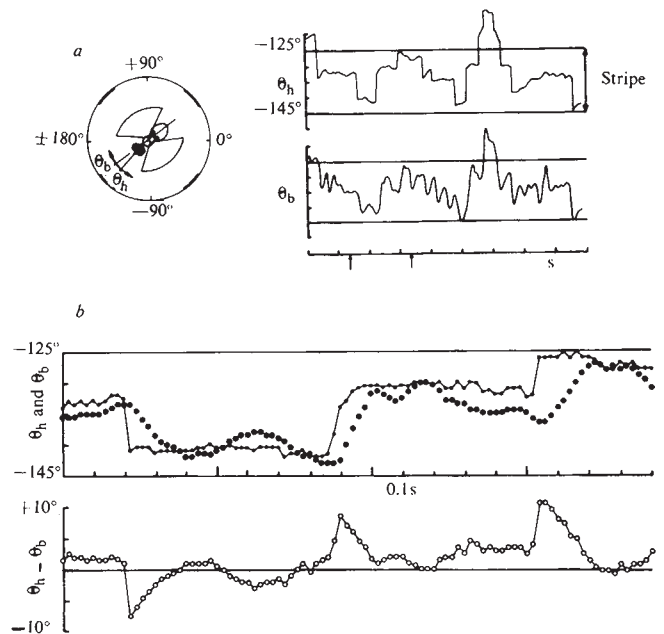


Fig. 1 *a*, Record of the movement of the head (θ_h) and body (θ_b) of a fly during a 9 s period in which it was fixating one of the four stripes of the drum, as shown on the left. The record shows 14 saccadic movements of the head. The sign convention used in all figures shows clockwise angles as negative. *b*, Part of the record from (*a*), between arrows on the time scale, at 10 times the temporal resolution. ●, Body position; ●, head position; ○, movements of the neck ($\theta_h - \theta_b$). Each point is the position on a single frame of film. It can be seen that between saccades the head position is kept stable in spite of body movements by active movements of the neck.

examples are the fixation of prey by mantids⁶ and by salticid spiders⁷, and compensatory eye movements in a moving visual surround by crabs⁸ and locusts⁹. Here I describe the head movements (and therefore, in insects, eye movements) made by blowflies during sustained flight. I show these to be of two kinds: rapid saccadic flicks in which the head takes up a new angle with respect to the visual surroundings, and stabilization movements which keep the angular position of the head almost constant, in spite of body movements of up to 10° in either direction.

Blowflies (*Calliphora erythrocephala*) were suspended on a pivot which allowed them to rotate freely but prevented forward motion. The pivot consisted of a pin revolving in a narrow glass tube, sealed at the base and partially sealed at the top to prevent lateral play. A second pin was waxed to the dorsal thorax, bent around the animal and joined to the pivot. Although nearly frictionless, the pivot does add an inertial load tending to reduce the rate at which the fly can change direction. Flying flies were placed at the centre of a 12 cm diameter white drum containing four equidistant black stripes, each 20° wide by 110° high. The drum was lit from above giving an average background brightness of about 200 cd $\times$ m⁻². The flies were filmed from above at 50 frames s⁻¹; the films were analysed frame by frame to give the angular positions in the horizontal plane of the head (θ_h) and body (θ_b) separately. A small piece of celluloid attached to the head made it possible to measure both angles to an accuracy of about 1°.

In a stationary drum blowflies flew in the direction of one or other of the four stripes for about 75% of the time, so that θ_b was usually within 20° of the centre of a stripe; shifts of fixation from one stripe to another were frequent. Similar behaviour has been demonstrated by Reichardt in *Musca* in an artificially produced “closed loop” environment^{4,10}. Although the fly's body points in the general direction of a stripe, its head movements are much more precise. Fig. 1 shows on two time scales the movements of head and body during part of a long bout of fixation of a single stripe. The following points

emerge. (i) The head fixates different positions within and just outside the stripe, and maintains them accurately in spite of movements of the body. In some records the body departed by as much as $\pm 7^\circ$ from its average direction of flight, whereas the head moved through only $\pm 2^\circ$. (ii) Changes in fixation point are accomplished by rapid saccadic movements of the neck (three are shown in Fig. 1b). These have amplitudes between 5 and 20° , and are complete in 20 to 40 ms. The head usually moves at at least 500° s^{-1} . (iii) After each saccade the body changes direction slowly, coming into line with the head again after 100 to 200 ms, though this is likely to be less without the inertial load of the pivot. The body then follows the same course as the head, but with less precision and some oscillation. (iv) The stability of the head between saccades is achieved by active neck movements in the opposite direction to the body movements. Thus in Fig. 1b the neck movements ($\theta_h - \theta_b$) are almost the mirror image of the body movements (θ_b) except during saccades.

The stabilization of the head between saccades is under direct visual control. This is demonstrated in Fig. 2 in which the drum is rotated; the head now accurately follows the drum movement between saccades as though "clamped" to the visual field.

When a fly has abandoned one stripe and is turning towards another, the behaviour of the head is similar to that during stripe fixation (Fig. 3). It makes rapid saccades with short (50 to 200 ms) intervals between them. The saccades tend to be more frequent than during stripe fixation, and are usually all in the same direction until another stripe is reached.

The combination of rapid saccadic eye movements followed by periods of relative stability of the fixation point appears to be a visual strategy common to both flies and man, and no doubt to many other visual animals. If certain kinds of perception such as pattern discrimination or the detection of external movement can only occur when the visual world is temporarily still, then it is necessary for a moving animal to have mechanisms for keeping the eyes in a constant optical relationship to the external world (stabilization), and for changing this relationship at intervals (saccades) as new information is required or locomotion shifts the scene.

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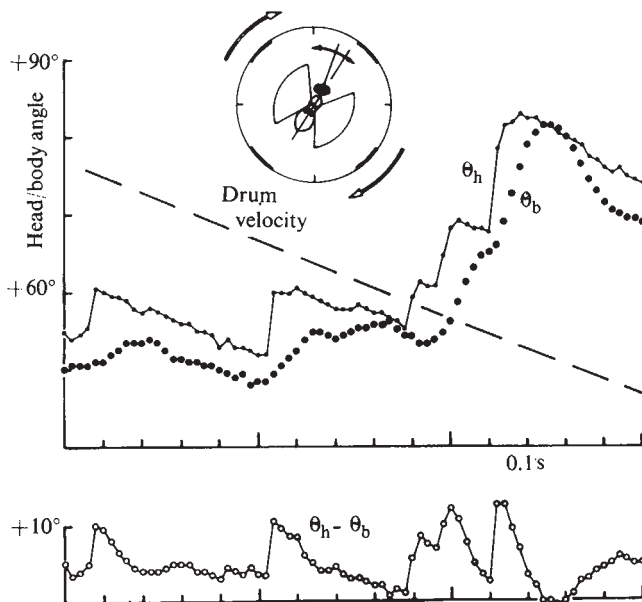


Fig. 2 Record of head and body movements, as in Fig. 1b, but in which the striped drum is moving clockwise at 20° s^{-1} . The dashed line indicates the drum velocity, though not the exact position of any one stripe. Between the five anti-clockwise saccades made by the fly, the head remains accurately stabilized on the moving pattern.

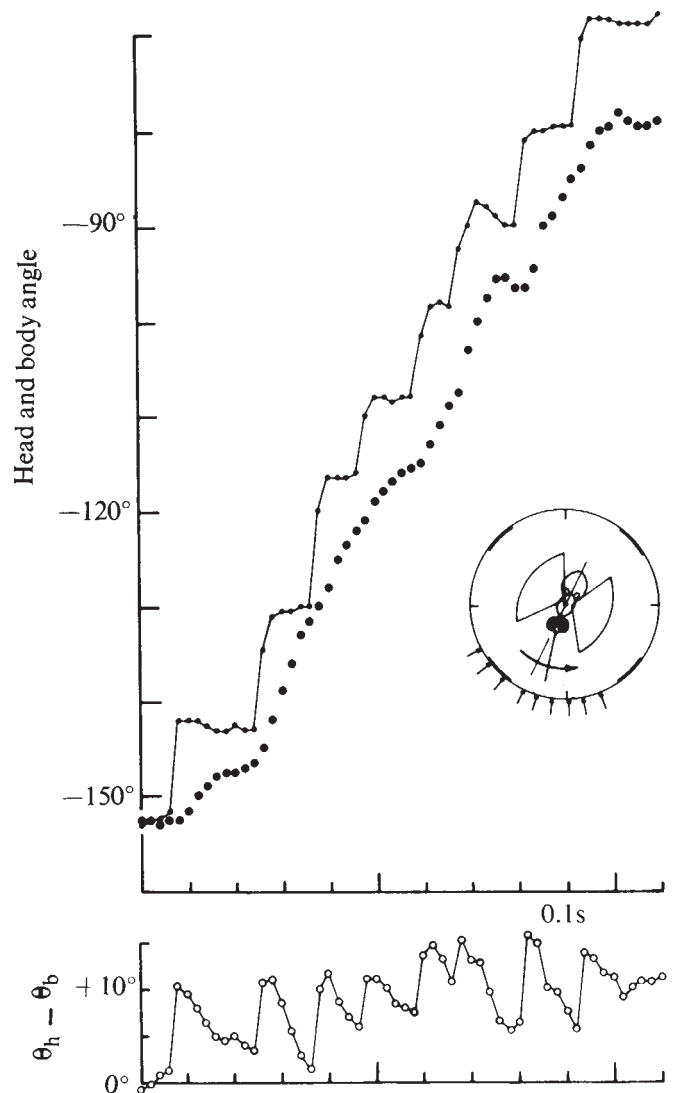


Fig. 3 Head and body movements during anti-clockwise turning when no stripe is being fixated. As in fixation, the head makes saccadic flicks with short periods of stability between them (arrows on inset). The drum is stationary.

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"Electromotor" Response in the Electric Fish *Eigenmannia* (Rhamphichthyidae, Gymnotoidei)

VARIOUS species of fish are known to emit and perceive weak electrical signals¹⁻⁴. Whereas the physiological mechanisms involved in the production and perception of such signals have been investigated in detail (ref. 3, pages 347 and 493), less is known about their behavioural relevance. Lissman² proposed and gave evidence for a role in object detection, by the distortion of the fish's own electrical field due to either dielectric or conducting objects within range. This idea has been supported by later work (refs 4-6 and personal communication from A. Kalmijn and R. Adelman). The study reported here was undertaken as a first effort to devise ways of measuring the performance of the object detecting system in respect to its discrimination of particular parameters such as distance, size and motion. A primary goal was to find methods based on built-in responses, rather than time-consuming conditioning procedures, which could be used on any individual on a given day to assess its skill in relation to species norms and would thus facilitate the quantification of changes in performance after nerve transections, brain lesions and such like.

Fish of the genus *Eigenmannia* hover in plant thickets during the day and swim into open water only at night⁷. When kept in aquaria without vegetation *Eigenmannia* readily hovers in bundles of plastic strips hanging from the water surface. When a plastic arrangement of this kind is moved gently *Eigenmannia* tries to maintain its relative position inside. To determine to what extent electrical object location could account for this behaviour the following experiment was performed.

A specimen of *Eigenmannia* (resembling the species *Eigenmannia virescens*) was blinded to exclude visual cues and placed in a bare all-glass aquarium 90×30×40 cm high. Three vertical 'Plexiglas' strips, 1.5 mm thick and 35 mm wide (see cross-section in Fig. 1), were hung at the centre of the aquarium reaching down into the water a few millimetres above the glass bottom. This arrangement was chosen after six blinded specimens tested in various arrangements of 'Plexiglas' strips were found to hover preferably with the nose close to one strip and at least one side of their body near another strip. This behavioural preference was met by the symmetrical arrangement shown in Fig. 1. After 2 d of restless swimming following the operation the fish began to hover inside the 'Plexiglas' arrangement close to the bottom, usually oriented as in Fig. 1. When the fish happened to be sufficiently stationary the entire 'Plexiglas' arrangement was slowly swung horizontally in a sinusoidal manner, parallel to the plane of the strips in order to minimize water turbulence. As the 'Plexiglas' arrangement began to swing, the fish responded by translating itself right and left, still hovering, by means of its constantly undulating anal fin which tilted left and right respectively. The fish thus maintained its position relative to the 'Plexiglas' strips for several cycles (Fig. 1) until it eventually swam away. Commonly it hovered and swung back and forth over periods of 30 s to 5 min.

To determine the extent to which mechanical cues of the swinging 'Plexiglas' arrangement could have accounted for this response, an identical arrangement was built with agar strips of conductivity equal to the aquarium water (2 kΩ cm⁻¹), agar recipe developed by Kalmijn and Adelman (personal communication). Whereas the fish usually avoided contact with the 'Plexiglas' strips it now frequently bumped into the agar strips. It rarely hovered in the neighbourhood of the agar arrangement and generally had to be prodded into it before experiments could start. When the agar arrangement was swung the fish did not respond and usually escaped when eventually hit by one of the strips (Fig. 1). This suggests that *Eigenmannia* perceived the movement of the previous

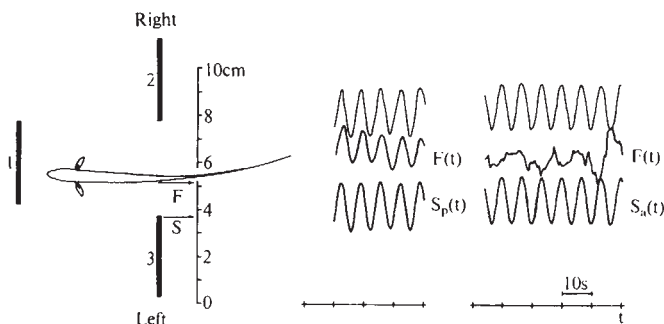


Fig. 1 Left, cross-section through experimental arrangement of three vertical strips, 1, 2 and 3, with fish hovering between strips 2 and 3. While the entire arrangement is swung right and left, its momentary position along the cm scale is recorded by the location of the inner edge of strip 3 (S). The position of the fish is recorded by the location of its left side body wall on the level of strips 2 and 3 (F). Right, two continuous records of S and F obtained by single frame film analysis. A simultaneous tracing of the inner edge of strip 2 is shown above each record. S_p , swinging 'Plexiglas' strips inducing strong response in the fish (gain=0.7, phase= $-\pi/10$ according to Fourier analysis of F and S_p at stimulus frequency). S_a , swinging agar strips of same conductivity as water fail to elicit a response (gain=0.1, phase= $-\pi$ at stimulus frequency) and cause flight as fish is hit twice at the end of the record.

'Plexiglas' strips electrically rather than mechanically. *Eigenmannia* thus follows electrically-detectable objects in a manner comparable with the way visual animals follow light patterns, for example, a beetle following the rotation of a striped cylinder. By analogy with this famous "optomotor response"¹⁰ one can call the behaviour in *Eigenmannia* an "electromotor response".

The electromotor response in *Eigenmannia* shares obvious advantages with the optomotor response by being non-habituating and thus easily repeatable. Experiments can be performed almost any time with the only restriction that the animal has to be hovering rather than restless. A healthy and well fed animal, however, can be expected to hover for at least a few hours every day, whereas specimens suffering from skin diseases, injuries or hunger are constantly restless and thus not available for experiments. As a further advantage experimental strategies and mathematical procedures, such as Fourier analysis, so far successfully applied in optomotor studies can also be applied to investigate the electromotor response and thus allow the quantification of the performance of the electric object detection system in terms of phase and gain values as functions of temporal and spatial frequencies.

Considering the sinusoidal movement of the 'Plexiglas' arrangement as input and the sinusoidal motion of the fish as output, one can determine the transfer function of this system by applying different input frequencies and measuring the gain and phase of the fish's response. As Fig. 2a shows, the fish maintains a high and constant gain over at least three octaves (0.025 to 0.2 Hz) whereas it follows with decreasing amplitude and increasing lag at oscillation frequencies greater than 0.2 Hz. This deterioration in performance, however, may be a consequence chiefly of the mechanical inertia of the fish rather than an overt sensory deficiency. As high frequencies of oscillation are applied, the fish tries to follow by stronger sideways undulations of its anal fin but never reaches a sufficient speed to follow the 'Plexiglas' strips over their total excursion.

In analogy to visual performances one would expect weaker responses as narrower 'Plexiglas' strips are chosen for the arrangement shown in Fig. 1 and that responses should finally cease as the threshold size of electrically-detectable strips is reached. A fish hovering in the swinging 'Plexiglas' arrangement responds to the reversal of its motion with a certain delay. This leads to a corresponding phase lag between the

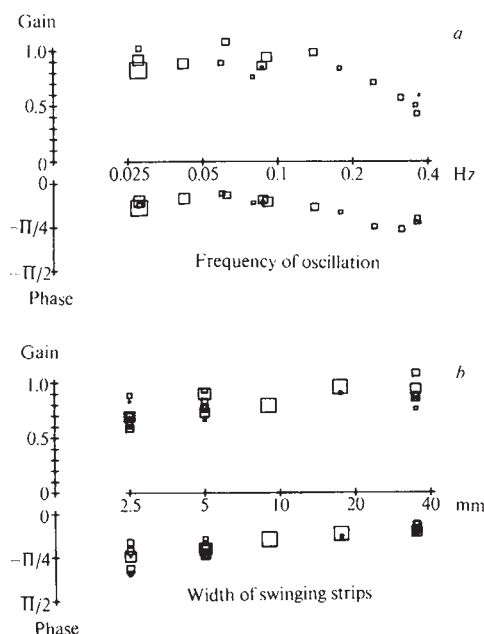


Fig. 2 Gain and phase of electromotor response in one *Eigenmannia* specimen as functions of frequency of oscillation (a) and width of swinging strips in 'Plexiglas' arrangement shown in Fig. 1. Experimental specifications: a, 'Plexiglas' strips 35 mm wide, 1.5 mm thick, peak to peak amplitude of oscillation 20 mm, inner distance between strips 2 and 3 35 mm; b, frequency of oscillation 0.1 Hz, peak to peak amplitude of oscillation 15 mm, inner distance between strips 2 and 3 30 mm, all strips 1.5 mm thick. Linear size of squares proportional to length of individual data record available for Fourier analysis, maximum length 120 s.

sinusoidal motion of the fish and the sinusoidal motion of the 'Plexiglas' strips. When narrower strips are chosen the fish's response is more delayed (Fig. 2b). Finally, at a strip width of 2.5 mm the lag may become so large that the fish almost collides with a swinging strip before reversing its motion. It thus seems that narrower strips have to approach and to withdraw over greater distances before the fish can decide in which direction it has to travel.

The measurements illustrated here were all obtained from one specimen which gave consistent results during several weeks of experiments and required frame by frame measurements of cine film, followed by semiautomatic digitization and computer calculation of phase and gain. To obtain a first estimate of individual variation four more specimens were tested with 2.5 mm wide strips at an oscillation frequency of 0.1 Hz. The mean phase and gain values found ranged between $-\pi/8$ and $-\pi/4$ and between 0.9 and 1.1 respectively and thus did not differ much from the corresponding values in Fig. 2b.

One may argue that a fish following the swinging 'Plexiglas' arrangement only escapes from the strip which approaches its side rather than follows the opposite strip as it moves away. However, when the fish happens to hover a few centimetres outside the arrangement, it still follows both the approach and the withdrawal of the strips, all of which lie to one side of the fish; it follows, to be sure, in a less stationary fashion and may lose "contact" after a few cycles. The 'Plexiglas' arrangement thus operates in a "push-pull" fashion when the fish hovers inside, which seems to account for the greater stability of its response.

Eigenmannia is thus able to control its distance from nearby objects of different conductivity than water. This mechanism should enable the fish to hold a certain position in a habitat of swaying plants without depending on visual or mechanical cues.

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Mercury and Other Metals in British Seals

INCREASING concern about environmental pollution has led to many investigations of heavy metals and their distribution in the sea, air and biological materials. There have, however, been few investigations of seals and here we give some preliminary results of analyses of British seals. Soft-tissue samples were taken from ninety-six seals (Table 1), both common seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*), from six sites around Britain between February 1968 and January 1972; these were collected during the course of long-term population studies. Results from some areas lack data on samples from the middle age-group as mature seals cannot be aged by external features but by examination of canine teeth^{1,2}. Data from East Anglia for both species were pooled because of small sample sizes. Brain and liver samples were obtained from most seals and kidney and teeth from about a third; all tissues were placed individually in uncontaminated polythene bags and stored at -20°C . The wet samples were digested in nitric acid and total mercury was estimated using a Perkin-Elmer 305 atomic absorption spectro-

Table 1 Geographical Distribution and Age Groups* of Seals Sampled

Area	Species	0-0.9	1.0-5.9	6-10.9	11-15.9	16-20.9	21-25.9
1. Wash	<i>P. vitulina</i>	9	1	2	1		
2. Scroby Sands	<i>H. grypus</i>	2 (3)	1 (4)	(1)	1 (1)		
	<i>P. vitulina</i>	2	3				
3. Farne Isles	<i>H. grypus</i>	5	2 (3)	1	2	2	1 (2)
4. West Scotland	<i>H. grypus</i>	(3)	(2)	(2)		(1)	
	<i>P. vitulina</i>	4	3	4	2		
5. Outer Hebrides	<i>H. grypus</i>	5		1		2	3
6. Orkney and Shetland	<i>H. grypus</i>	4 (9)	3 (5)	1		(1)	

* Seals were aged to the nearest whole year, but have been grouped to simplify the Table.

Figures in parentheses indicate additional seals from which tooth samples only were taken.

photometer (AAS). Cadmium in soft tissues was estimated by AAS using a flame technique; teeth, collected from 1968 onwards, were dissolved in nitric acid and analysed on a Perkin-Elmer 303 AAS for lead, cadmium, copper, chromium and zinc.

The results, summarized in Table 2, showed that there were regional variations in mercury concentrations in the brain, Hebridean values being higher than those of the Farnes; the results from the Hebrides and Farne Islands were significantly greater than those from all other regions. These variations may be a consequence in part of the differential passage of mercury compounds from the blood to brain tissue, the "brain-barrier" effect having previously been demonstrated in toxicological studies³. Methyl-mercury, which is extremely toxic⁴, can easily pass this barrier^{4,5} compared to inorganic and most organic mercurials.

Except for the common seals from West Scotland, the concentrations of mercury in the brain did not increase with age (Fig. 1), suggesting that a ceiling level is reached early in life. Mercury concentrations in liver were positively correlated with age (Fig. 2) in five of the six areas, the exceptions being Shetland seals in which levels were lower than those from all other areas. Analysis of combined data from all areas gave a correlation coefficient of 0.78 ($P < 0.001$). Analysis of variance on the transformed data for all areas showed that

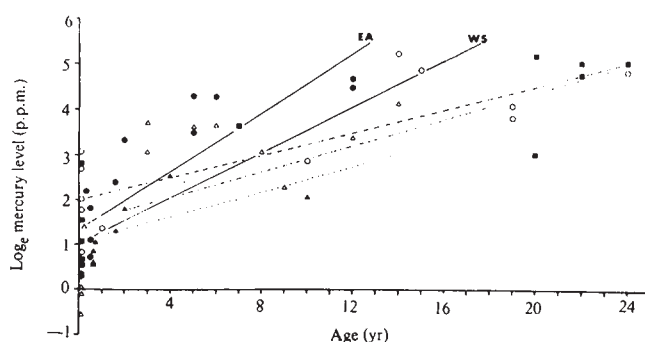


Fig. 2 Mercury in seal liver 1971-72 (p.p.m. wet weight total mercury). —●—, East Anglia (EA), regression line: $y = 0.340x + 1.282$, correlation with age: $r = 0.845$, $P < 0.001$; ---○---, Farne Islands, $y = 0.132x + 2.021$, $r = 0.832$, $P < 0.001$; - - -■- - -, Outer Hebrides, $y = 0.150x + 1.467$, $r = 0.886$, $P < 0.001$; —△—, West Scotland (WS), $y = 0.254x + 1.036$, $r = 0.726$, $P < 0.01$; ...▲..., Shetland, $y = 0.146x + 0.993$, $r = 0.689$, $P > 0.05$.

vitulina constituted 88% of the seals from the East Anglia/West Scotland group, and seals from all other areas were *H. grypus*, any conclusions about differences between the species must

Table 2 Mercury and Cadmium Concentrations in British Seals (1971-1972)

Areas 1 and 2 East Anglia			Area 3 Farne Islands			Area 4 West Scotland		Area 5 Outer Hebrides			Area 6 Shetland		
Hg brain	Hg liver	Hg kidney	Hg brain	Hg liver	Cd kidney	Hg brain	Hg liver	Hg brain	Hg liver	Cd kidney	Hg brain	Hg liver	Cd kidney
0.4 ±0.2 (13)	29.9 ±38.7 (15)	13.7 ±0.2 (2)	0.7 ±0.2 (7)	84.2 ±68.9 (7)	5.3 ±4.0 (5)	0.4 ±0.1 (8)	30.1 ±18.2 (9)	0.7 ±0.2 (6)	113.0 ±68.0 (6)	11.6 ±7.5 (4)	0.3 ±0.1 (8)	4.9 ±3.6 (8)	2.2 ±2.0 (8)

Mean values ±SD in p.p.m. wet weight.

Figures in parentheses are the number of samples. Cadmium values for all brain and liver samples were less than 1 p.p.m. Pup and foetus values (involving areas 3, 4 and 5 only) have been omitted from the calculations, as they represent maternal pollutants; a mother and her pup/foetus can be considered as one and the same marine-feeding unit.

there was an overall significant difference between some of the slopes ($F = 2.979$, $P < 0.05$), due to the East Anglia and West Scotland regressions having greater slopes (that is, higher build-up of mercury in liver) than the other three areas. Although P .

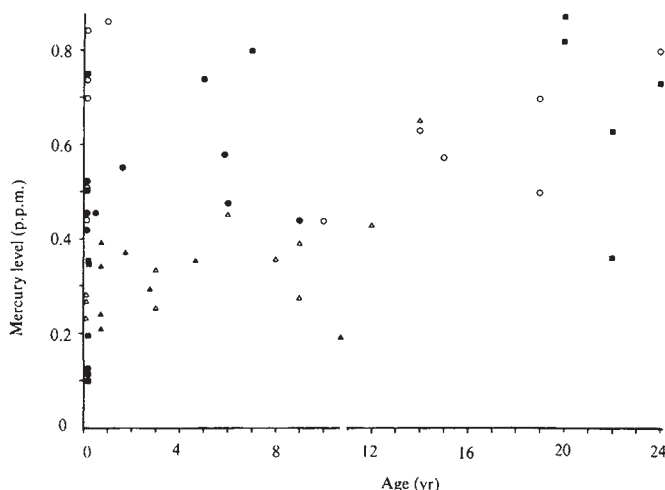


Fig. 1 Mercury in seal brain 1971-72 (p.p.m. wet weight total mercury). Correlation of concentration with age: ●, East Anglia, $r = 0.459$, $P > 0.1$; ○, Farne Is., $r = -0.068$, $P > 0.1$; ■, O. Hebrides, $r = 0.545$, $P > 0.05$; △, West Scotland, $r = 0.778$, $P < 0.01$; ▲, Shetland, $r = -0.537$, $P > 0.1$.

remain tentative at present, as the age spectra of each group differed, and there were more older seals in the samples of *H. grypus*. Further extensive sampling is needed to establish if the straight line model for Log_e of mercury accumulation with age is adequate.

Table 3 Grey Seal *Halichoerus grypus*—Pup/Mother Ratios of Mercury and Cadmium Residues (1971-1972)

Area	Amount of mercury in brain	Amount of mercury in liver	Amount of cadmium in kidney
3. Farne Islands	1.02 (5)	0.12 (5)	0.08 (3)
5. Outer Hebrides	0.57 (5)	0.06 (5)	0.05 (3)

Mean values p.p.m. net weight.

Number of observations (pup/mother pairs) are shown in parentheses.

Cadmium was found in comparatively large quantities (up to 22 p.p.m. wet weight) in all kidneys (Table 2), the concentration tending to increase with age ($P < 0.001$). Brains and livers, however, contained less than 1 p.p.m.

Mercury and cadmium in pup tissues varied in relation to maternal levels (Table 3) and in general reflected the tendency for some metals to show an age accumulation in certain tissues. Other studies have shown that the placenta constitutes a barrier to the passage of most mercury compounds except methyl mercury⁶ which passes into the foetus easily and may reach a concentration comparable with that in the mother⁷.

Table 4 Metal Concentrations in Grey Seal Teeth

Area	n	Lead	Zinc	Chromium	Copper	Cadmium
1. } East Anglia	20	36.7 ± 7.5	103.9 ± 56.4	19.9 ± 7.9	4.9 ± 6.2	3.9 ± 0.8
2. } Farne Islands	15	30.6 ± 7.7	142.4 ± 63.6	14.7 ± 6.5	6.9 ± 5.3	3.5 ± 0.5
3. } Outer Hebrides	13	32.7 ± 10.5	145.8 ± 66.9	14.3 ± 3.9	4.1 ± 1.8	3.8 ± 0.7
4. } Inner Hebrides (West Scot.)						
5. } Orkney and Shetland	15	37.0 ± 10.4	85.2 ± 45.3	18.1 ± 8.4	4.7 ± 4.7	5.2 ± 2.5
Correlation with age of seal	63	P < 0.001 (-ve)	P < 0.001 (+ve)	P < 0.001 (-ve)	P > 0.1	P < 0.05 (-ve)

Mean ± SD p.p.m. dry weight¹¹.

Lead, zinc and chromium were found in molar teeth, with cadmium and copper in smaller quantities (Table 4). Analysis of the data in relation to age showed a progressive decrease in concentrations of lead, cadmium and chromium; young seals contained higher lead levels than older individuals ($P < 0.001$), the chief change occurring at about 5–7 yr of age. This may reflect the fact that, initially, enamel, cement and dentine are laid down together in molar teeth, but after about 5 yr only small amounts of biologically inert cement are deposited², so that relatively little extra material is incorporated into the tooth. Metal concentrations will tend to remain stable after 5 yr or decrease slightly with the slow accretion of cement and may therefore reflect, to some extent, the metal concentrations encountered during the first 4 or 5 yr of life. Zinc levels, however, showed a reverse trend, increasing with age. Regional comparisons of metals in teeth revealed no clear pattern, but cadmium and lead decreased in the order Orkney and Shetland, East Anglia, Hebrides, Farne Islands. There was no significant difference between lead residues from Scotland and East Anglia.

Our preliminary study suggests the following: 1, Mercury reaches a ceiling level in brains at about 12–18 months; residues in man reach similar levels and also show no correlation with age^{5,8}. 2, Mercury tends to accumulate in the liver with age, reaching fairly high levels. 3, Cadmium accumulates in the kidney, as it does in man⁹, where it is known to occur in higher concentrations than in other organs¹⁰. 4, There do not seem to be regional differences in metal concentrations in teeth, but some metals were found in greater quantities in younger seals than in old ones, probably related to the pattern of tooth growth. 5, There are regional differences in the amounts of mercury in the tissues, but there does not seem to be any relationship with possible agricultural or industrial pollution.

Our results demonstrate the importance of taking age into account when studying the amounts of metals in biological tissues. Conclusions based on small samples, when the age of the animal is unknown, can be misleading.

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Egg Taints: Association with Trimethylamine

MILLER¹ has described brown-shelled eggs with a characteristic taint produced in various parts of the United Kingdom during autumn 1971 and early 1972; this problem has occurred extensively in the UK over the same period in earlier years but it was much more apparent in 1971–1972.

Brown-shelled eggs from the eight commercial flocks of birds which we have investigated contained from 6% to 27% of eggs described as "fishy" or "crabby", similar to those of Miller¹. No tainted white shelled eggs have been reported to us.

Birds from a typical large outbreak were examined and 20 birds of a single strain with "bad breath" were selected since this condition had been associated with tainted eggs^{1,2}. Using the identical diet and individual caging the eggs produced were examined individually and those regarded as significantly tainted by all the testing panel (3 or 4 subjects) were used for investigation of the compound responsible for the taint. Tainted eggs (1,400 g, pH 7–8) were freeze dried and the distillate (1,100 ml.) collected at -198°C . The condensate was melted, acidified (pH 3) and freeze dried to give crude amine hydrochlorides (60 mg); gas chromatography on an alkaline support³ showed that the characteristic odour was coincident with the peak corresponding to trimethylamine and this identification was confirmed by mass spectrometry. Tainted eggs, including examples from Miller's experiments¹, contained more than $1.0\text{ }\mu\text{g g}^{-1}$ of trimethylamine whereas untainted eggs contained less than $0.1\text{ }\mu\text{g g}^{-1}$; most of the trimethylamine ($\sim 95\%$) was present in the yolk. The odour threshold concentration which we have determined for trimethylamine in water ($0.0005\text{ }\mu\text{g g}^{-1}$) is below that in tainted eggs.

On our own control diet these selected birds produced no tainted eggs; when trimethylamine hydrochloride (100 mg day^{-1}) was fed to nine of the twenty birds, they all produced characteristically tainted eggs containing trimethylamine ($3\text{ to }40\text{ }\mu\text{g g}^{-1}$) within six days.

Repetition with fifteen 52-week-old brown birds with no record of producing tainted eggs, showed, however, that only two of these birds produced eggs containing trimethylamine (10 to $40 \mu\text{g g}^{-1}$).

The production of tainted eggs is therefore, like bad breath^{1,2}, associated only with certain individual birds. Gas chromatography/mass spectrometry on samples of breath from the birds described by Miller¹ has shown that the major odorous constituent is dimethyl sulphide and aqueous solutions of dimethyl sulphide at concentrations of 10^{-14} to $10^{-15} \text{ g ml}^{-1}$ have odour characteristics extremely similar to those of "bad breath". We have not, however, been able to confirm a direct relationship between these two effects.

These preliminary results indicate that a complex situation is involved. A dietary source of trimethylamine or of a trimethylamine precursor is necessary and we have been able to reproduce the phenomenon in selected birds by feeding a diet containing particular batches of rape seed meal. This meal contained less than $1 \mu\text{g g}^{-1}$ of free trimethylamine, and is not therefore an immediate source of preformed trimethylamine, which must arise by an, as yet, undefined route. The coincidence of at least two factors, in the bird and in the diet, for the production of this situation is clearly necessary and is probably the origin of the sporadic nature of the problem. There is no evidence that fish meal is involved. Further investigations are in progress.

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Temperature Coefficient (Q_{10}), Seed Germination and Other Biological Processes

A COMMON method of comparing rates of reactions or processes in biological systems is the use of the temperature coefficient, or Q_{10} , the ratio of the rates of a reaction or process at $(T+10)^\circ\text{C}$ and $T^\circ\text{C}$. James¹ says of the Q_{10} : "As it is readily calculated, easily understood, and carries no theoretical implications, it remains a useful and favourite method of recording the temperature relations of complex biological processes". But because Q_{10} values of different processes tend to fall within certain limits, they are often used to indicate types of reactions that might be occurring.

The basis for this depends upon the description of biological processes in terms of the Arrhenius law^{2,3}. This predicts that the Q_{10} values of many chemical reactions obeying the law will be within the range 2 to 3 at room temperature (justifying the van t'Hoff rule) and that Q_{10} values will fall gradually with increasing temperature. Several biological processes, whether seemingly governed by individual chemical reactions or whether describing gross physiological processes of tissues or populations, do seem to obey this law^{2,4}, but in many biological processes it is normal to find that Q_{10} values fall with increasing

temperatures at a rate faster than would be predicted from the Arrhenius equation^{1,2}.

One approach to this discrepancy between chemical and biological kinetics is to consider Q_{10} in relation to the total temperature range of the process. In chemical kinetics the relative constancy of Q_{10} for reactions following the Arrhenius law is dependent on the slow rate of change of the product of T and $(T+10) \text{ K}$. In a physiological process there is a temperature range which is comparatively narrow and beyond which the process or reaction does not occur. The Q_{10} is therefore calculated for a process that rises from zero to a maximum and then falls again to zero within a temperature range probably not exceeding 40 to 50°C . Often consideration of Q_{10} is restricted to the increasing phase only. Any Q_{10} for a biological process calculated with the minimum temperature as the lower temperature limit (when the rate is just not detectable) has a value of infinity; any Q_{10} calculated with the maximum temperature as the upper temperature limit has a value of zero. Under normal circumstances, and over the whole temperature range in which the process occurs, the value of Q_{10} for that process must fall from infinity to zero with increasing temperature. During the phase of increasing rate of reaction, when it could be argued that the effects of enzyme inactivation due to low or high temperature are possibly not acting, there may be a range of temperature over which Q_{10} is relatively stable, but this is not necessarily the case.

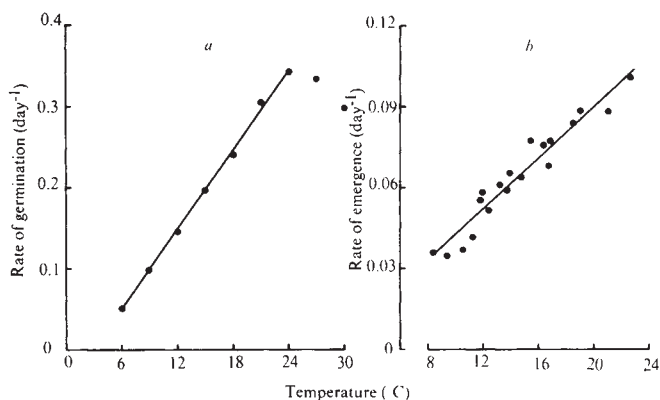


Fig. 1 *a*, Rate of germination against temperature for carrots in the laboratory; *b*, rate of emergence against mean soil temperature for carrots in the field.

Exceptions are to be found, for instance, in values of Q_{10} derived from germination data. Kotowski investigated the rates of emergence from sand of several vegetable species⁵ and found that Q_{10} values were higher at lower temperatures and lower at higher temperatures with mean values usually in the range 2 to 3.5. He concluded that the time rate of the production of seedlings at different temperatures agreed well with the van t'Hoff temperature coefficient ranging from 2 to 3. But the Q_{10} values given by Kotowski for all species were as low as 1 to 1.5 between 25 and 30°C and as high as 5 to 8 between 8 to 11°C and 4 to 8°C .

Harrington⁶ followed the germination on paper of a range of vegetables and Leach⁷ the emergence from sterile soil of five crop types. Commenting on the results Harrington stated: "Kotowski found Q_{10} s of 4 to 12 between 4 and 8°C ; Leach's results are comparable when the Q_{10} is calculated from his data and the results of this (Harrington's) research are similar, averaging 11.26 between 0 and 5°C . The Q_{10} s steadily decrease to an average Q_{10} of 0.84 between 30 and 35°C . This shows how extremely temperature dependent are the reactions involved in germinating seeds."

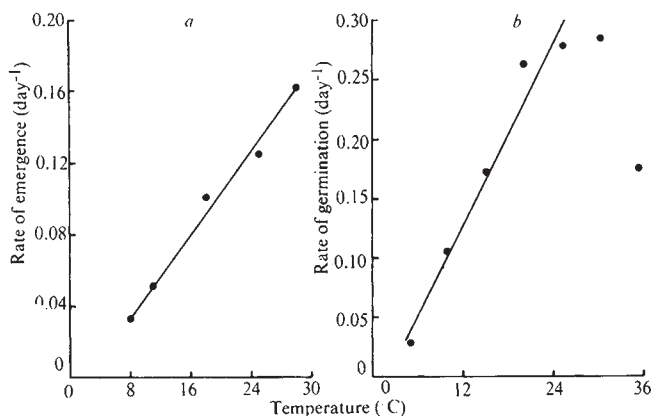


Fig. 2 Rates of emergence and germination of carrots at constant temperature. *a*, Emergence from sand⁵; *b*, germination on paper⁶.

Recent work⁸ has shown that for carrot seeds in a temperature range of almost 20° C the relationship between rate of germination and temperature in the laboratory, and between rate of emergence and mean soil temperature in the field, is linear to a good approximation. The data (Fig. 1) were obtained in 1972, but allowing for problems associated with extreme moisture stress similar results have been obtained using a number of different carrot seed lots in previous years. Similarity of the Q_{10} values calculated for different temperature intervals with those of Kotowski⁵ and Harrington⁶ suggests that their results might also show linear relationships if plotted in this way, and in spite of the small number of points on the graphs, this did seem to be

the case for a restricted temperature range (Fig. 2). Plots of rate of germination or emergence against temperature for a limited number of other species have also given good approximations to linear relationships (Fig. 3*a, b, c*). But in some cases where there seems to be a good linear relationship (Fig. 3*d*) an analysis of variance shows that there are significant quadratic and cubic components in the variation due to temperature, indicating that the curve is probably sigmoid over this temperature range.

The occurrence of a linear relationship does not necessarily suggest that there is a single dominant rate-limiting process governing germination that is itself linearly related to temperature. It is probable that the rate of a biological process is determined by a complex of reactions and processes each independently affected by temperature⁴, and a more likely explanation of the relationships is that in some cases the rate-limiting reactions involved combine to give, fortuitously, a linear dependence of rate on temperature over a relatively wide temperature range.

Whatever the underlying principles behind the linear relationship may be, these relationships have considerable potential use for the development and justification of cropping programme models based on heat unit or linear temperature/growth concepts⁹ and provide a convenient means for comparing the germination rates of different seed lots (Fig. 4).

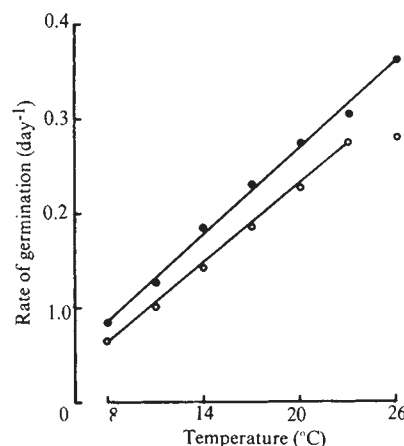


Fig. 4 Rate of germination against temperature for two carrot seed lots. The difference between the slopes was significant ($P < 0.05$).

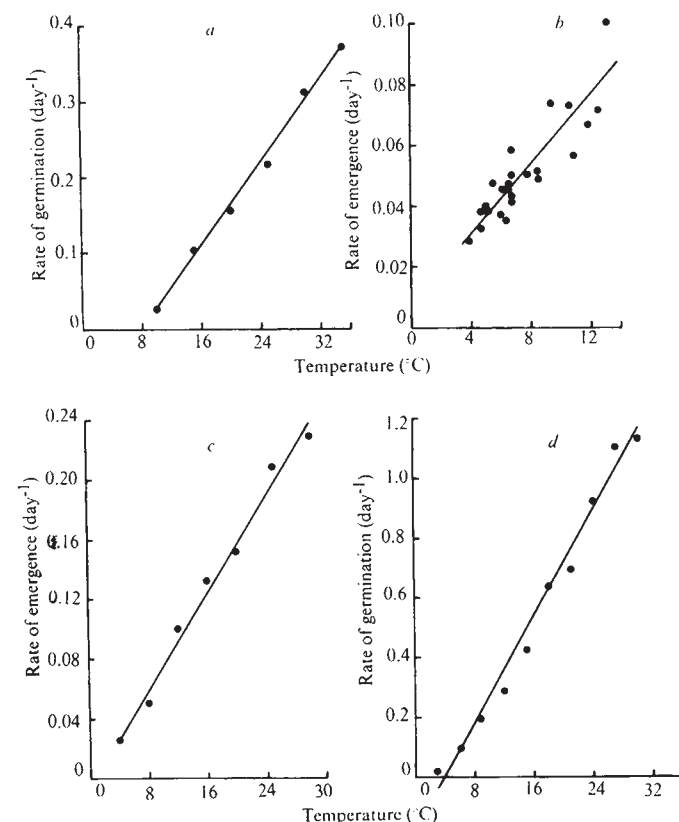


Fig. 3 Rates of emergence or germination against temperature or mean soil temperature for: *a*, snap bean on paper⁶; *b*, spring oats in soil (based on the time to appearance of the crop)¹⁰; *c*, wheat from sterile soil⁷; *d*, cauliflower on paper.

Concentration on the Q_{10} as an easy and convenient means of expressing such relations can be misleading and, perhaps more important, can obscure simple and potentially useful relationships.

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BOOK REVIEWS

Machines of the Past

The Computer from Pascal to von Neumann. By Herman H. Goldstine. Pp. x+378. (University of Princeton: Princeton, New Jersey, January 1973.) £6.25.

It is rare to be able to say of a new book that it is the first in its field, but such is the case with this production of Herman Goldstine. There has, up to now, been no volume devoted exclusively to the history of computers and computing, although of course many books published in the last twenty years have had a preliminary section dealing with matters historical.

Dr Goldstine has a unique title to write a history of computers because he was one of the people involved in the earliest days of the electronic era. Furthermore, as practically the last representative of the famous Princeton Institute for Advanced Studies group, Goldstine is performing a service in establishing precisely the role which the Institute played in the development of electronic computation. Goldstine himself seems to have doubts whether the Institute's role has been sufficiently recognized. From a now completely impartial position within the field, I find this one of the few statements I would dispute. Wherever electronic computation is talked about, and wherever expert groups gather, the Institute is usually credited, even now, with pioneer efforts in the field of true electronic computation.

Trained originally as a mathematician and physicist, Goldstine worked with the Moore School group on the ENIAC and its successors, transferred to the Institute for Advanced Study, where he worked with the late John von Neumann, succeeded von Neumann as Project Director, and then, with the closure of the Institute's computer project, moved to industry, which he considers the most active area now. He has held distinguished posts, mostly with the IBM Corporation, where he was Director of Mathematical Science at the Thomas J. Watson Research Centre, Director of Scientific Development of the Data Processing Division, and is now enjoying an honoured position as an IBM Fellow.

The book has three main parts—the first effectively historical, the second concerned with the great upsurge of computer activity in World War II,

and the last dealing with activities at the Institute for Advanced Study after the conclusion of hostilities. There is also an Appendix. The opening chapter on the historical background is, perhaps, slightly skimpy, although the author has made an original contribution in his discovery of the work of Wilhelm Schickard who designed and built in 1623 a machine capable of automatic addition and subtraction and semi-automatic multiplication and division. Unfortunately, Schickard's actual device is lost, although some indications of its method of operation still exist, and the IBM Corporation, as part of its general historical and cultural activities, has made a version of the machine which is illustrated in the book. Schickard clearly anticipated the work of Blaise Pascal who is usually credited with the invention of the first adder-subtractor in 1642. Leibniz, whose multiplier dates from about 1690, was also anticipated, although it would seem that Schickard's methods of semi-automatic multiplication and division were quite different from the step wheel conceived by Leibniz.

As usual in books on computation, the author of this one spends some time on the English genius, Charles Babbage. Dr Goldstine appears to take a position which has been espoused by several recent writers that Babbage was a misanthrope and a crank. Here I disagree with the author, who has not, perhaps, read some of Babbage's works on the nature of industry in depth. These show that, far from being a man who continually devised new solutions to computational problems and abandoned his partially constructed devices without completion, he devoted much of his energy to the improvement of manufacturing techniques and technology in England. It is true that the technology of the time was not adequate to realize Babbage's conception, but, far from "ending it all with a bare bodkin", Babbage "took arms against a sea of troubles", and if he did not end them, he at least made some impact on the scene.

These are minor criticisms, however, for the book goes on to a fascinating discussion of the origin and provenance of the ENIAC, and includes many sidelights, generally unknown, on the wrangling and disharmony which eventually disrupted the Moore School team. The discussions about the work

at the Institute for Advanced Study are, of course, seminal, and, apart from brief mentions in obituary notices of John von Neumann, have not hitherto been revealed. They are an important contribution to the history of computation. Perhaps inevitably, portions of the book, and particularly the Appendix, which deal with developments outside of the United States, are less satisfactory than the rest. For example, the attribution to Atanasoff on page 123 of the development of binary methods in digital computation completely ignores the claims of E. Williams Phillips, the English actuary whose contributions are well documented. Yet again, perhaps too much importance is attached to certain evanescent developments such as the Forrester-Williams storage tube. Perhaps more important is the omission of reference to the work of K. D. Tocher, the actual inventor of micro-programming despite the urgent protestations of some later rediscoverers. These things, however, are minor blemishes.

The book is first-rate: it is written in a style that all can understand, and where advanced mathematical techniques are mentioned, sufficient background is provided for even the lay reader to understand what is going on. Goldstine has a lucid style, and it is greatly to be hoped that in due course we shall see other productions from his pen.

ANDREW D. BOOTH

Observations on the Sun

Solar Activity: Observations and Predictions. Edited by Patrick S. McIntosh and Murray Dryer. Pp. xv+444. (MIT: Cambridge, Massachusetts and London, November 1972.) \$17.50.

WITH this publication the MIT series on *Progress in Astronautics and Aeronautics* reaches its thirtieth volume. Arising out of a 1970 conference held in Huntsville, Alabama, the book comprises twenty-five papers reviewing current knowledge of solar activity, its effects on the interplanetary and terrestrial environments, and the possibilities of predicting such phenomena. Compared with the material found in some such conference products, this work effectively avoids the pitfalls of over-specialization and subjective research opinions, a success perhaps

attributable to the non-specialist format requested from contributors and to the inclusion of extra papers to fill gaps in the conference material.

The papers fall into four sections, starting with "Solar Observations and Theories", moving out through the "Interplanetary Medium" to consideration of "Geophysical Responses to Solar Activity" and completed by studies of "Progress in Forecasting Technology". J. W. Evans leads off with his "Introductory Review of Solar Activity", a highly competent and concise account of solar phenomena and nomenclature for the newcomer to the field, spoilt only by some lack of attention to numerical detail and descriptive clarity in places. Further papers in section 1 divide equally between observations of large and small scale solar magnetic field distributions and of flare particle, radio, ultraviolet and X-ray-line emissions, rounded off by Sturrock's résumé of magnetic flare models. Chief omissions are adequate discussion of hard X-ray observations (an important flare diagnostic) and explicit description of optical flare characteristics other than inference of flare fields from H α filtergrams.

Continuing through the papers on interplanetary and geophysical aspects, the reader encounters a continuing high quality and density of information. Particularly valuable to the newcomer are the reviews of the solar wind by Dryer and Cupperman and of magnetospheric particles by Paulikas. Despite all this material, however, a shadow of the stamp-collecting era remains looming over the science of solar activity. Application of games theory (Smith) and of Markov chains (Mogilevsky) gives "successful" activity predictions for only a few days, little better than suitably "accurate" and "skilful" subjective classification of active regions. (Predictions of heliographic locations of activity are more satisfactory.)

Thus, barring a spectacular improvement in these techniques or a conceptual breakthrough in activity theories, McIntosh and Dryer's editorial anticipation of the military, astronautic and ecological benefits to accrue from activity predictions must be, at best, long-term ideals. Viewed, however, as a unifying account of solar activity, their book can be recommended to all.

JOHN C. BROWN

Critique in Palaeontology

Models in Paleobiology. By Thomas J. M. Schopf. Pp. vi+250. (Freeman, Cooper: San Francisco, 1972.) \$9.50.

AT last we have been provided with a thought-provoking text on invertebrate palaeontology. Tom Schopf is to be congratulated not only on having the vision and temerity to question the

motivation and direction of palaeontological enquiry but also for producing this timely panoramic critique at a comparatively moderate price.

The volume comprises ten essays on various aspects of palaeontological analysis and synthesis grouped under the headings of (1) "Models in Paleobiology", (2) "Morphology", (3) "Population and Evolution", and (4) "Distribution". They result from a 1971 symposium on models in palaeobiology jointly sponsored by the Paleontological Society and Geological Society of America at their annual general meeting in Washington DC. The styles of the presentations vary from author to author but each is introduced by a pertinent précis by Schopf. Thus no matter how complex and abstruse some of the articles may at first seem, Schopf has succeeded in ensuring that all contributions are intelligible even to those geologists who suffer from minimal and/or inappropriate scientific backgrounds. This is no mean feat since the discussions liberally impinge on the cognate sciences of evolutionary palaeontology, biogeochemistry and statistics.

Schopf's implied secondary theme that all too frequently the last decade has witnessed an explosion in unquestioning and uncanalized palaeontology is hard to refute. In assembling these papers he has effected a heroic job which ought to revitalize the science and its utility. Even now there are deficiencies which are a salutary reflexion on the inadequacies and inappropriateness of our scientific trainings. Too often we are trained to see the trees at the expense of seeing the wood. The advances quoted in this volume are frequently simple and derive from using the methodology of a cognate science rather than a large body of complementary subject matter. The basic deficiency underlying all the themes is our bland neglect of the physiological parameters which have a profound influence on both biogeochemistry and ecology, which thereby affect evolutionary palaeontology and culminate in the difficulties which arise in statistical analysis as a result of cumulative omission. This does not mean that statistics should be ignored as unsound, but that it should be treated with the reverent constructive caution so admirably displayed by Simberloff. For, as Eldredge and Gould so rightly, but damningly, imply, we tend to report those things for which we have explanations while ignoring those aspects which embarrass us. This is perhaps epitomized in Dodd and Schopf's truism that the biogeochemical data available to us are woefully inadequate as the geochemical results have seldom tested the intimate relationships which would have been required had the

enquiries rested on sound biological principles. This can easily be seen to result from the fact that all too frequently undergraduate geologists align themselves with either the physical sciences or the biological sciences but seldom both. Thus, quite apart from the intrinsic value of the variety of approaches clearly analysed, the volume makes salutary reading for those concerned with both educational and applied aspects of geology and the earth sciences.

This book should be compulsory reading for all geologists and earth scientists who direct departments and assess research proposals. It will provide palaeontologists with many happy hours of brain-teasing, stimulation and self-assessment. It should intrigue the curious undergraduate, absorb the graduate student, and infuse panic in the hearts of those palaeontographers who have got stuck in a rut. No geology department library can afford to be without this volume, for its intellectual applications are not limited to palaeontology alone. This tome has achieved for palaeontology an analogy of what the Geophysical Laboratory, Washington DC, contributed to petrology some decades ago.

J. A. E. B. HUBBARD

Vibrations in Fluid

Sound, Structures and Their Interaction. By Miguel C. Junger and David Feit. Pp. x+470. (The MIT: Cambridge, Massachusetts and London, October 1972.) \$27.50.

THERE are many books on acoustics and many on vibrations. Some even consider the radiation of sound from vibrating surfaces and others the vibration induced in a structure by an impinging sound wave. There is, however, no book devoted to the interaction between structures and the surrounding fluid. At best a chapter or two in existing texts might be devoted to particular aspects of the interaction. The majority of written work on this topic is spread through the technical literature, and not a small portion of this is classified because of its underwater naval applications. Dr Junger himself has been responsible for several significant developments in underwater acoustics.

This textbook is the first book to draw together much of this material and to present it in a coherent way. The book arose from the need, at post-graduate level, of a text on the interaction between a vibrating structure and the surrounding medium. The interaction becomes most important with dense fluids such as water, but there are cases in which even the weaker coupling with air is important. The results will also be important for containment structures such as gas cooled

nuclear reactor circuits. The book is largely theoretical but does cover cases and conditions which are of interest in practical problems. Many valuable results which are scattered through the literature are brought together in this book. Naturally these include Dr Junger's own contributions—for example his classic work on the radiation of sound from cylindrical shells vibrating in a dense fluid is well presented. This is of value from the point of view of far field radiation but also from the feedback effect giving the damping of the vibrating structure.

The basic acoustic theory of sound sources is clearly presented and developed to multiple arrays and radiation from vibrating surfaces. The authors then go on to consider radiation from vibrating surfaces submerged in a fluid and complete this with a comprehensive treatment of cylindrical and spherical shells. The basic theory of the scattering of sound by rigid bodies is followed by a chapter dealing with elastic scatterers and reflectors. This latter topic is of growing importance and has not been treated in the standard textbooks. The approach throughout is deterministic but asymptotic techniques for high frequency acoustical, vibration and interaction problems are presented.

There is no mention of the growing amount of work on the statistical energy method of analysing structuro-acoustic interaction problems. Perhaps this could form a second book in the series on structural-acoustic coupling.

In conclusion I consider that this book fills the need for a textbook which presents in a comprehensive way all the acoustic and vibration theory which is required to arrive at a deterministic type of solution to interaction problems. It will be of great value to postgraduate students and research and development workers in this field of growing interest.

B. L. CLARKSON

Mercury Poisoning

Mercury in the Environment: an Epidemiological and Toxicological Appraisal. Edited by Lars Friberg and Jaroslav Vostal. Pp. 215. (CRC: Cleveland, Ohio, 1972.)

GOING along with the current advancement in chemical industry, the water pollution caused by mercury is gradually increasing. Since the human hazard of mercury, well known by the name "Minamata disease", exists in Japan where the first mysterious poisoning occurred, this problem has been taken up as an urgent matter at an early stage, more so than in other parts of the world.

While this is becoming an increasing worldwide problem, the book *Mercury in the Environment* is timely and the contents useful.

This book first introduces the up-to-date methods of analysis of mercury, both organic and inorganic, and also introduces transport and transformation of mercury in nature, and possible routes of its exposure. Secondly, it discusses metabolism and especially about distribution, retention and excretion in the living body, as well as including the latest information about symptoms and signs of the poisoned individual. To discover the normal content of mercury in tissues, urine and blood of the body, the toxic effect of mercury compounds, how they are formed, and the amounts which cause intoxication are being minutely investigated. Lastly, they comment on the genetic effects of mercury.

Gathering all possible literature concerning environmental pollution and damage caused by mercury, this book comprehensively covers the current information and knowledge of the mercury problem.

TADAO TAKEUCHI

Electromagnetic Waves

Theory of Ionospheric Waves. By K. C. Yeh and C. G. Lui. Pp. xiv + 464. (Academic: New York and London, December 1972.) \$24.

THIS is essentially a monograph concerned with the propagation of electromagnetic waves in an ionized gas, featuring those aspects of propagation that are of most concern to the radio physicist. Thus the medium is treated almost exclusively by means of its fluid approximation and equivalent dielectric characteristics, and propagation is treated by means of an inevitable succession of stages of increasing complexity: without and with a background magnetic field, without and with a significant temperature of the plasma, without spatial variations and then with gradual variations and random, scattering variations, of background parameters.

Most of the topics treated cannot help but be familiar to readers of other texts on ionospheric radio propagation or on waves in plasmas. What will make this text distinctive and perhaps preferable for some such readers is its own particular choice of path through well-trodden territory. An outline of the ionosphere is given for initial stage-setting, along with a forewarning of the dyadic notation that permeates later pages. A purported review of electromagnetic theory is hardly what it calls itself, but is in fact a much needed summary of concepts and techniques applicable to the excitation and propagation of electromagnetic energy in dielectric media. Two appendices to the volume, concerned with the method of steepest descents and radiation fields from localized sources, might well have

formed an integral part of this review. Plasma properties are then introduced, first via such processes as screening and longitudinal waves, and only later through the more usual route, at least for radio physicists, of the general spectrum of waves with its various special cases: high frequencies, electron and ion whistlers, hydromagnetic frequencies, and so on. The usual geometric optics, WKB approximation, coupled equations, and scattering approximations are followed by a helpful discussion of nonlinear wave processes, a topic too often avoided.

The closing chapter of the volume treats atmospheric waves (except for tides) at ionospheric heights. While it is good for its length, it seems to be more of an afterthought than a correspondingly rounded discussion: a condescension to the book's title, perhaps, or to its inclusion in a series of geophysical monographs. Still, those who buy the volume for its electromagnetic content will find themselves exposed in this chapter to some of the geophysical content of the ionospheric regions that attract their attention, and exposed also to the mathematical linkages—if not to the underlying physical linkages—that unite this geophysical content to the electromagnetic content. It will be a useful exposure for many, and will set the book apart from others of its ilk.

C. O. HINES

The Botanic Tongue

Botanical Latin: History, Grammar, Syntax, Terminology and Vocabulary. By William T. Stearn. Pp. xiv + 566. (David and Charles: Newton Abbot, February 1973.) £6.50.

IT is good to see that *Botanical Latin* (more usually known as "Stearn") is again available. Comparison of the first and second editions shows that the pagination is unaltered and the number of additions and corrections small. Unfortunately the second edition has been printed on thicker paper than the first edition and this makes it both heavier and less convenient to handle; but this is a minor drawback.

Anyone who has to read or write botanical Latin or needs to know the precise meaning of a host of technical descriptive terms will find this book invaluable. The introductory fifty-six pages can be read with pleasure and profit by any botanist; these are followed by a section dealing with grammar, hardly light reading but essential to the average taxonomist. The third section, "Syntax and other matters", contains much of general interest, including an extensive list of place names and colour terms. The main part of this section is devoted to examples of diagnoses and descriptions

of organisms ranging from algae and fungi to flowering plants. These provide for almost any imaginable situation which might face the describer of a new species when trying to comply with the requirements of the International Code of Botanical Nomenclature for a Latin diagnosis. It also exemplifies the use of Latin as a living modern language devoid of nationalism. Some of its more recent developments are to be seen in the chapter on chemical reactions and tests, though many of the older chemical terms are, of course, derived from pharmaceutical works. The vocabulary of 171 pages, which forms the bulk of the concluding section, is full of information not readily obtainable elsewhere.

As one whose formal training in Latin consisted of six months dreary grind which culminated in passing "Little-go" at the second attempt, I find Dr Stearn's book invaluable. I notice also that some of my colleagues in the Classics department dip into it with interest, and one of them has remarked that if the Romans had sent telegrams they would have developed this sort of Latin. This, I think, provides an objective estimate of the conciseness and clarity which botanical Latin has attained.

It is pleasing that in the seven years that have elapsed between the first and second editions the price has only risen from £5.25 to £6.50. This is a small price to pay for a concise history of systematic botany and of the development of the Latin alphabet and much else besides. T. G. TUTIN

Interpreting Fossils

The Meaning of Fossils: Episodes in the History of Palaeontology. By Martin J. S. Rudwick. Pp. 287. (Macdonald: London; American Elsevier: New York, 1972.) £6.

THERE are probably some university departments of geology in Britain which even now do not offer their students any formal course of instruction in the history of the subject. Too often in the past it has been thought sufficient that the lecturer in palaeontology, stratigraphy, petrology or what you will, should preface his first-year course with a statement on the development of knowledge in that branch or at best devote his introductory lecture to a review of progress of ideas in it over the past hundred years or so. It is often doubted whether students are at that early stage equipped with sufficient basic knowledge of geological terminology to appreciate the analysis which is being made. The dilemma remains that at later stages of the undergraduate course, there is increasing pressure on curriculum time and such addition as the history of the subject is hardly likely to receive any priority.

Dr Rudwick states that his book had

its origins in his course of lectures at Cambridge arranged primarily for undergraduates reading history and philosophy of science, but also attended by geology students. The subtitle of the work, rather than its title, indicates its scope and five "episodes" in the history of palaeontology have been chosen for exposition spanning the period from the sixteenth to the mid-nineteenth century.

Beginning with Conrad Gesner's contribution "On Fossil Objects" (1565), Dr Rudwick first develops the theme of the progression from the classification of "fossils" to the first glimmerings of an interpretation of the forms resembling living organisms, and in doing so he introduces the work of a number of the naturalists of Western Europe and relates it to the contemporary philosophical climate of the Renaissance period.

Each of the following chapters raises one or more of the problems which confronted the naturalist in his search for the nature and significance of fossils through the following centuries. The seventeenth century with its tentative approach to the organic theory and the problems arising from the position and altitude at which fossils are found provides the link with ideas on stratification. There follows the period with the constraint imposed by the theologically based limit on geological time, and later an exposition of Charles Lyell's ideas of what the author describes as a "steady-state" model of Earth-history.

The detailed work of the comparative anatomists at the beginning of the nineteenth century is emphasized in reference to their contribution to the various evolutionary theories which followed. The limitations of the palaeontological record as a means of confirmation of the Darwinian theory almost form the climax. Alongside this is the account of the stratigraphical search back into the older rocks for early forms of fossils and for evidence of some beginning of life itself.

Dr Rudwick has been very successful in conveying to the reader the manner in which successive problems in palaeontology were conditioned by the general thinking of the period. To be fully understood, the plausibility of each theory must be judged only in the light of the whole background of knowledge and philosophical temper of the time at which it was propounded.

Writing for two groups of students who have approached the subject from very different directions has imposed special constraints on the author. He has met the challenge with a concise and lucid account which is well illustrated. There is a glossary of about a hundred geological and palaeontological terms; but it has been assumed that the geological student has ready access to a

dictionary for definition of such adjectives as teleological, anti-heuristic, and so on.

By reading this book the student of geology, whether specializing in palaeontology or not, could well develop a permanent interest in the broader historical aspects of the subject, and the excellent bibliography which is provided will be invaluable to him for further reading. J. M. EDMONDS

Mammalian Biology

Mammalogy. By Terry A. Vaughan. Pp. viii+463. (W. B. Saunders: Philadelphia, London and Toronto, 1972.) £6.20.

THE first fourteen chapters of this attempt at a single volume presentation of the biology of mammals comprise an adequate, and in places lively, account of mammalian form and species diversity. The orders are tackled systematically and each chapter is well illustrated. The remaining eight chapters deal with such generalia as ecology, zoogeography, behaviour, reproduction, metabolism and temperature regulation, water regulation, acoustical orientation and general conservation problems. These chapters are rather less than totally adequate, but perhaps this shortfall is the inevitable outcome of an attempt to cover too vast a field in too little space.

Mammalogy is written for the American market and aimed at college and university students. European students may experience difficulty in coping with the American vernacular, names for scientific names are not invariably given. The reader may be puzzled by the rather arbitrary choice of topics singled out for elaboration in the latter part of the book. For example, why is a whole chapter (which, incidentally, happens to be very good) devoted to acoustical orientation and specializations of the ear, but only one page to olfactory communication? Being on average nineteen pages long, each chapter allows only a glimpse at the field and there are, naturally, disappointing omissions. In the chapter on behaviour a discussion on the mechanism bringing about observed behaviour and a fuller treatment of the problems concerning innate and learned behaviour would have been welcome. The final chapter on mammals and man is very short and adds little to what every student knows—that the future survival of many mammal species is in the balance.

Expensively produced and well illustrated this book can hardly be recommended as a "must" for European students who tend not to study subjects like mammalogy *per se*, but their teachers may find it a convenient, though limited, reference book.

D. MICHAEL STODDART

CORRESPONDENCE

Nuclear Defence

SIR,—I supported wholeheartedly your editorial "France should not test" (*Nature*, **243**, 50; 1973), but I think that perhaps you were a little unfair in your remark that the French Government would be well advised to admit that its *force de frappe* is no more than an illusion.

I would, of course, agree that for its advertised purpose as a contribution to the defence of Europe against possible Soviet aggression, its short range and small size make it utterly insignificant if the United States is involved, and, of course, like our nuclear force, more likely to induce preventive attack than to act as a defence if the Russians could be sure that the Americans would not be involved.

I am surprised, however, that you have taken its advertised object at its face value, rather than as the window dressing which it seems to me to be. It is surely easy to believe that the real concern of the French general staff is still, as it has always been, with Germany rather than with Russia. This is not even any reflexion on the general well-meaningness of the present West German Government. Governments have changed in this respect in the past, and anyway, general staffs concern themselves with what is possible rather than with what governmental intentions are likely to be. Without nuclear weapons France could offer little resistance to a revanchist Germany and might well doubt the willingness of the United States to destroy such a Germany on behalf of France, and in doing so, destroy their main bulwark in Europe against the USSR.

Ludicrously inadequate as it would be in face of a Russian attack, the *force de frappe* is in both range and power entirely adequate as a deterrent to a Germany which does not possess such nuclear weapons. One could well blame the French general staff for taking the same narrow and limited view of their country's interests as is taken by all other national general staffs, but I think that it is unfair to blame them for suffering from illusions.

Yours faithfully,

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Reaching for the Stars

SIR,—I feel obliged to respond to the two articles "Eye on the Future" and "The Search for Signals from Extraterrestrial Civilizations" (*Nature*, **241**, 363 and 379; 1973).

The first of these, an excellent brief review of the Project Cyclops report*, ends by questioning the cost and value of the search for extraterrestrial intelligence: "The scale of expenditure suggested for Cyclops, however, runs at four times the total annual expenditure of the British Science Research Council. Perhaps the question is whether it is more efficient or more satisfying to spend \$600 million a year asking the stars for, say, a cure for cancer or to spend the same sum on cancer research on Earth." These peculiar comparisons ignore the difference in the potential support base and imply that a cure for cancer would be a major, if not the only, benefit of interstellar contact. Ideally Cyclops would be an international effort; but even excluding this possibility, the \$600 million/year (during the construction phase only) is only 8 per cent of the 1966 US space budget and 20 per cent of that for 1973.

Conceivably a cancer cure could be discovered out of interstellar contact (assuming our biochemistry is not too different from theirs), but is only one of a host of potential benefits, many of which may be far more important. Any civilization we contact will be at least as advanced as we and probably much more so. In all probability they will have faced and solved many of the problems that beset the world today such as pollution, depletion of natural resources, population control and the assurance of continued genetic evolution in a compassionate society, to name only a few.

Present theories of galactic evolution and of the origin and evolution of life support the belief that intelligent civilizations have existed in our Galaxy for a few billion years. Interstellar communication may therefore also have existed for aeons, and our first contact could put us in touch, not with another isolated civilization, but with this galactic community. We would then have access to a galactic heritage of knowledge that might include, in addition to a five billion year pictorial record

* Copies available from John Billingham, NASA/Ames Research Center Code LT, Moffett Field, California 94305.

of the universe, the Galaxy, and its life forms, some insight into the common cause of intelligent life and its future role. Interstellar contact may well mean childhood's end for the human race. With what other outcome can this be compared?

In the second article James C. G. Walker analyses the consequences of using a type of search strategy that was known to be inadequate before the Cyclops study began. Walker rejects omnidirectional beacons on the grounds that "the power requirement of an isotropic call signal detectable at 100 light years is approximately equal to the world's present total power consumption". He then assumes beamed beacons that illuminate any particular receiver for a short fraction of the time and, not surprisingly, arrives at very pessimistic figures for probable search time. The Cyclops study assumes that the total cost of achieving contact should be minimized by balancing the costs of transmitting and receiving. This leads to receiving antennas several km in diameter, which can be realized as filled phased arrays. Omnidirectional beacons are then quite feasible and with these the search times are greatly reduced as indicated in Table 1.

Table 1 Search Times for Galaxies, using Walker and Cyclops Strategies

Average separation of communicative civilizations	Duration of search	
	Walker strategy	Cyclops strategy
24 light yr	14 centuries	1 d †
240 light yr	14 million yr	1 yr
2,400 light yr	140 aeons ‡	370 yr

† Needed to get complete sky coverage; otherwise 8 h.

‡ Should be reduced, galactic disk is only about 1,500 light yr thick in solar neighbourhood.

A Cyclops receiving system with an effective clear antenna aperture of 5 km would detect in 1,000 s a coherent signal having an incident flux of 5 photons s^{-1} m^{-2} §. It would detect a 16 megawatt omnidirectional beacon at 100 light years or a 1,600 megawatt one at 1,000 light years range. Even this latter beacon radiates less than one four-thousandth of the world's present power

§ Since these are 1.5 GHz photons an optical system of equal power sensitivity would have to be able to detect one photon s^{-1} /hundred thousand square miles.

consumption from fossil fuels, and could be powered with a single modern nuclear plant. Man will qualify for participation in the galactic community when he is willing to invest his share in a balanced system, rather than expecting the other civilizations to do the whole job.

Yours faithfully,

B. M. OLIVER

Plus ça Change

SIR,—Important decisions about the future development of atomic power must frequently be made by people who do not necessarily have an intimate knowledge of the technical aspects of reactors. These people are, nonetheless, interested in what a reactor plant will do, how much it will cost, how long it will take to build and how long and how well it will operate. When they attempt to learn these things, they become aware of confusion existing in the reactor business. There appears to be unresolved conflict on almost every issue that arises.

I believe that this confusion stems from a failure to distinguish between the academic and the practical. These apparent conflicts can usually be explained only when the various aspects of the issue are resolved into their academic and practical components. To aid in this resolution, it is possible to define in a general way those characteristics which distinguish the one from the other.

An academic reactor or reactor plant almost always has the following basic characteristics: (1) It is simple. (2) It is small. (3) It is cheap. (4) It is light. (5) It can be built very quickly. (6) It is

very flexible in purpose ("omnibus reactor"). (7) Very little development is required. It will use mostly "off-the-shelf" components. (8) The reactor is in the study phase. It is not being built now.

On the other hand, a practical reactor plant can be distinguished by the following characteristics: (1) It is being built now. (2) It is behind schedule. (3) It is requiring an immense amount of development on apparently trivial items. Corrosion, in particular, is a problem. (4) It is very expensive. (5) It takes a long time to build because of the engineering development problems. (6) It is large. (7) It is heavy. (8) It is complicated.

The tools of the academic-reactor designer are a piece of paper and a pencil with an eraser. If a mistake is made, it can always be erased and changed. If the practical-reactor designer errs, he wears the mistake around his neck; it cannot be erased. Everyone can see it.

The academic-reactor designer is a dilettante. He has not had to assume any real responsibility in connexion with his projects. He is free to luxuriate in elegant ideas, the practical shortcomings of which can be relegated to the category of "mere technical details". The practical-reactor designer must live with these same technical details. Although recalcitrant and awkward, they must be solved and cannot be put off until tomorrow. Their solutions require manpower, time and money.

Unfortunately for those who must make far-reaching decisions without the benefit of an intimate knowledge of reactor technology and unfortunately for the interested public, it is much easier to get the academic side of an issue than the practical side. For a large part those

involved with the academic reactors have more inclination and time to present their ideas in reports and orally to those who will listen. Since they are innocently unaware of the real but hidden difficulties of their plans, they speak with great facility and confidence. Those involved with practical reactors, humbled by their experiences, speak less and worry more.

Yet it is incumbent on those in high places to make wise decisions, and it is reasonable and important that the public be correctly informed. It is consequently incumbent on all of us to state the facts as forthrightly as possible. Although it is probably impossible to have reactor ideas labelled as "practical" or "academic" by the authors, it is worth while for both the authors and the audience to bear in mind this distinction and to be guided thereby.

Yours faithfully,

H. G. RICKOVER

This previously unpublished comment on the practical problems of nuclear power was originally written in June 1953 but is thought still to be relevant today.—Editor, *Nature*.

Announcements

International Meetings

June 13, **Radioisotopes in Haematology** (General Secretary, The British Institute of Radiology, 32 Welbeck Street, London W1M 7PG).

June 14, **X-ray Image Intensifier Systems** (General Secretary, Institution of Electronic and Radio Engineers, 8 Bedford Square, London WC1B 3RG).

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